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بسم الله الرحمن الرحيم

Comparative Theriogenology

Camels, Horses, Cattle, Buffaloes, Sheep and Goats

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Preface

This book gives a thorough account of Theriogenology in camels, horse, cattle, buffaloes, sheep and goats for veterinary students studying reproduction. Chapter 1 – 4 give an account of comparative anatomy and physiology of reproductive organs (Chapter 1), how to examine it, either manually per rectum or via ultrasound, and how to perform clinical pre breeding checks of each species (Chapter 4). It also describes how to reproductively manage the estrous cycle including follicular dynamics, estrous behavior, endocrine control and ways to manipulate and synchronize estrus.

Chapters 6 – 10 give a background in pregnancy and parturition. The subjects covered include development of the conceptus, endocrine control, pregnancy diagnosis tips for practitioner, including clear ultrasonograms of early pregnancy in each species (Chapter 6), and a description of the different stages of parturition (Chapter 8). The various problems which occur during pregnancy such as embryonic and fetal losses, uterine torsion, vaginal prolapse, abortion and dystocia are covered in Chapter 7 and 9, Chapter 10 describes the post partum period, its normal course, complications, diseases and how to deal with them.. Chapter 11 covers the functional anatomy and physiology of the genital system, puberty, breeding soundness examination and infertility in male animals. Chapters 12 entails assisted reproductive techniques such as AI, embryo transfer, IVF and cloning.

We wish to thank Dr. FA Sobyil, Dr. A Al-Hawas, Dr. AF Ahmed, Dr. SA Osman and Dr. M Tharwat for their kind support during the processing of this book. Moreover, we would like to thank the director of Qassim University, the Dean of College of Agriculture and Veterinary Medicine and the Head of Department of Veterinary Medicine of Qassim University for their relevant help in handing out such scientific books through Qassim University.

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List of Abbreviations

Abbreviation	Meaning
ABD	abdominal diameter
ABP	androgen binding protein
AI	artificial insemination
Am V	amniotic vesicle
AV	artificial vagina
AVD	Amniotic vesicle diameter
BPD	biparietal diameter
BSA	bovine serum albumin
BSE	breeding soundness examination
BVD	bovine viral diarrhea
BW	body weight
CAM	chorioallantoic membranes
CCP	corpus cavernosum penis
CEM	contagious equine metritis
CHD	chest diameter
CIDR	controlled internal drug release
CL	corpus luteum
COCs	cumulus oocyte complexes
CR	conception rates
CRL	crown-rump length
CYC	cyclic
DF	dominant follicle
DPBS	Dulbecco's phosphate buffered saline
EBD	eye-ball diameter
eCG	equine chorionic gonadotropin
EHV	equine herpesvirus
ELISA	enzyme-linked immunosorbent assay
EPF	early pregnancy factor

EVA	equine viral arteritis
EYC	egg yolk citrate
EYGB	egg yolk- glucose- bicarbonate.
EYSM	egg yolk- skim milk.
FCS	fetal calve serum.
FGA	fluorogestone acetate
FSH	follicle stimulating hormone
GA	gestational age
GCT	granulosa cell tumor
GnRH	gonadotropin releasing hormone
GT	genital tubercle
hCG	human chorionic gonadotropin
HSD	hydroxysteroid dehydrogenase
IGF	insulin-like growth factor
IM	Intramuscular
IVC	in vitro-culture
IVF	in vitro-fertilization
IVM	in vitro-maturation
IVP	in vitro-production
KIL	kidney length
LH	luteinizing hormone
MAP	medroxyprogesterone acetate
MG	melengestrol acetate
MOET	multiple ovulation embryos transfer
NCY	non-cyclic
NNR	non-return rate
OMD	omasal diameter
OPU	ovum pick-up
Ovsynch	synchronization of ovulation
PAG	pregnancy associated glycoprotein
PCR	polymerase chain reaction
PGF2α	Prostaglandin F2α
PGFM	13,14-dihydro-15-ketoprostaglandin F2α
PLD	placentome diameter
PMNs	Polymorphnuclear cells
PMSG	Pregnant mare serum gonadotrophin
PRID	progesterone releasing intravaginal device
PRL	Prolactin

PSPB	pregnancy-specific protein B
RFM	retention of the fetal membranes
RUL	ruminal length
SCNT	somatic cell nuclear transfer
T4	Thyroxin
TCFY	tris-citric acid -fructose-yolk
TE	total estrogen
THD	thorax height diameter
UTD	uterine diameter

List of Terminology

Term	Definition
Abortion	Fetal death and expulsion
Anestrus	No estrus signs
Caslick's operation	Partial suturing of the vulva lips
Cold shock	Injury to sperm caused by a rapid decrease in semen temperature
Dystocia	Difficult birth
Echogenic	A structure causing a marked reflection of the ultrasound beam
Endometrial cyst	Cystic dilatation of the endometrial glands
Endometritis	Inflammation of the endometrium
Epididymitis	Inflammation of the epididymis
Fetal Maceration	Septic death of the fetus
Fetal Mummification	Aseptic death of the fetus
Fetotomy	Cutting of the dead fetus into small parts to get it out the birth canal
Freemartin	A female born co-twin to a male
fremitus	Vibratory sensation of the blood flow through the middle uterine artery during pregnancy
Hermaphroditism	Presence of both sex organs in one animal
Hydrallantois	Excessive fetal fluid within the allantoic membranes
Hydramnios	Excessive fetal fluid within the amniotic membranes
Hydrometra	Accumulation of fluid in the uterus

Hydrosalpinx	Accumulation of fluid in the uterine tube
Hyperechogenic	Showing increased echogenicity
hypoechogenic	Showing decreased echogenicity
Nulliparous	A female has never given birth before
Orchitis	Inflammation of the testis
Ovarian cysts	Overgrowth of the ovarian follicles
Ovarian hydrobursitis	Adhesions and accumulation of fluid in the ovarian bursa
Ovarian inactivity	Small, static, non-function ovaries
Pluriparous	A female gave more than one birth before
Pneumovagina	Air suction into the vagina
Pyometra	Accumulation of pus in the uterus
Salphingitis	Inflammation of the uterine tubes
Silent Heat	unobservable estrus signs in a normally-cyclic animals with active ovaries
Vaginal adhesions	Occlusion of the vaginal passage

CHAPTER 1

Comparative Anatomy and Physiology of the Female Reproductive Organs

The topography of female genital organs seems to be important for clarifying some of the physiological and pathological findings, and handling of the clinical cases. The female reproductive organs of ruminants and equines consists of a pair of ovaries that produce egg cells or ova and uterine tubes where fertilization occurs and which carry the fertilized ovum to the uterus where growth of the fetus takes place. The cervix separates the uterus from the vagina or birth canal, where the sperms are usually deposited.

The comparative anatomy and images of the reproductive organs in farm animals are shown in Table (1) and Figs. (1-8).

1.1. Reproductive Organs of female Camels

The reproductive organs of the female camels are found cranial to the borders of the pelvic cavity showing different degrees of descending into the abdominal cavity.

1.1.1. Ovary

The ovary is attached to the broad ligament by a well-defined tough ligament which extends from the hilus of the ovary to the tip of the matching uterine horn. The left ovary is commonly more cranio-ventral in position than the right ovary. Both ovaries are enclosed within a fold of the mesosalpinx known as the ovarian bursa, the apex of this bursa forms a large circular orifice within which lies the fimbriae of the uterine tube. Mature follicles and current corpora lutea project from the main contour of the ovary and give it a more lobular form (Figs. 1A, 2A). The ovary consists of two major parts: the cortex and the medulla. The ovary is entirely enclosed by a tunica albuginea except in the area of the hilus. Follicular activity takes place in the cortex and ovulation can occur anywhere on this surface. However, as camelids are induced ovulators, i.e. under natural conditions they only ovulate in response to mating, there is no cyclical appearance of a corpus luteum in non-mated females. Corpora lutea are therefore only present in the ovaries of recently bred, aborted or pregnant females.

Follicular activity is dominated by 4 types of follicles, namely: small growing follicles, mature follicles, regressing follicles or over-large, anovulatory follicles. As the follicular waves overlap with each other, several generations of follicles may be present at the same time. The small growing follicles are visible on the surface of the ovary as small slightly raised vesicles measuring between 2 - 4 mm, whereas the mature pre-ovulatory follicle measures between 13 – 20 mm and is spherical, turgid, with a thin clear translucent wall and protrudes clearly from the ovarian surface. The appearance of regressing follicles depends on the stage of regression. At the start of regression, the follicular wall becomes thick and opaque and the diameter decreases slowly until the follicle recedes into the ovary itself. Large anovulatory follicles are present in about 50% of non-mated females and their size and appearance can be highly variable (Fig. 1A). They vary in size from 25 - 60 mm and may have a thin or thick, opaque wall and contain either serous or haemorrhagic fluid with various amounts of fibrin. It seems that both ovarian sides (left and right) are equally functioning. Left and

right ovaries were approximately equal in length (means 3.21 cm vs. 3.39 cm, respectively) and width (means 2.59 cm vs. 2.63 cm, respectively). Growing, mature, and overgrown follicles are observed in frequencies of 88.7%, 21%, and 12.9% of both ovaries. Presence of overgrown follicles seemed; at least in some cases; a physiological process, where they are found with other functional structures.

The corpus luteum forms after ovulation, which occurs 24 - 48 hours after mating. The ovulating follicle collapses at ovulation and then the follicular cavity fills with blood to form a corpus haemorrhagicum. Luteinization of the corpus haemorrhagicum occurs within 4 - 5 days and gives rise to a corpus luteum. Regression of the corpus luteum occurs between 10 - 12 days after a sterile mating or just before parturition in the pregnant camel. The corpus albicans, originating from the regression of the corpus luteum of pregnancy is hard, white or grey in colour and has no blood vessels on its surface. Corpora albicantia of different sizes (5 – 12 mm in diameter) can remain on the surface of the ovary of the female for a long time. In one of the author's studies, corpora lutea were found in 22.6% of examined cases in abattoirs. The corpora lutea were found in pregnant and non-pregnant genital organs. Single corpora lutea of the non-pregnant genitalia are found on the left and right ovaries in frequencies of 55.6% and 44.4%, respectively. The mean maximum diameter of corpus luteum was smaller in the non-pregnant animals (1.37 cm), compared to that of pregnancy (2.04 cm). The CL may be with or without central cavity.

1.1.2. Uterine tubes

The uterine tubes play an important role in storage of sperms, fertilization and early embryonic development. Unlike other mammals though the uterine tubes are enlarged at the uterine end and this unique arrangement allows prolonged storage of large numbers of spermatozoa. The length of the uterine tubes measures between 17 and 28 cm. The isthmus is less coiled than the ampulla, and the fimbria lies within the bursa at a short distance from the ovary (Fig. 3A). Each uterine tube opens into the uterine horn via a narrow orifice at the summit of a protuberant papilla which can be as much as 3 - 5 mm in height. These papillae are very muscular and present a sphincter muscle at its apex. It possibly plays a role in the selective transport of fertilized embryos.

1.1.3. Uterus

The uterus in all camelidae is bicornuate. The left horn is larger than the right horn demonstrating variable degrees of asymmetry (Figs. 1A, 4A). In nulliparous females the uterus is small and can be found entirely within the pelvic cavity, whereas in mature non-pregnant females it is located in the abdominal cavity at the level of the 5^{th}, 6^{th} and 7^{th} lumbar vertebra. The non-gravid uterus has a short body of only 2 – 3.5 cm in length and the horns vary between 6 – 10 cm (right) and 8 – 15 cm (left) (Fig. 4A). In Bactrians, the body of the uterus can be as long as 8.5 – 9.5 cm in length and the right and left horns measure between 6 – 8 cm and 8 – 12 cm respectively. The endometrium of the uterine body and horns contain no caruncles and the uterine glands are simple, branched and tubular, and open in the surface of the epithelium (Fig. 4A). In the gravid uterus the left uterine horn, in which the fetus implants, becomes noticeably distended at around 1.5 months of pregnancy and is almost double its size by 2 months at which time the uterus hangs into the abdominal cavity. By 150 days the diameters of the gravid and non-gravid horns are nearly 4 times their original sizes, the endometrium increases in size and the glands become more numerous. The non-involuted post-partum uterus hangs over the brim of the pelvis. The uterine wall is thickened, edematous, and in the early postpartum period the uterus contains a small amount of blood. The uterus returns to its normal non-pregnant position between 20 – 45 days after parturition.

1.1.4. Cervix

The cervix of the dromedary has between 3 and 6 annular muscosal folds but its consistency does not differ significantly from that of the uterus, which makes it very difficult to identify by rectal palpation (Fig. 5A). In camels the cervical canal varies between 4 – 6 cm in length and 3.5 – 6.1 cm in diameter during follicular activity, but these decrease slightly during ovarian inactivity. The cervix protrudes caudally in the vaginal cavity forming a fornix of variable depth (1 – 1.5 cm). The size of the protruded vaginal portion of the cervix and the actual position within the vaginal cavity varies from animal to animal and the appearance of the external cervical os varies according to the stage of the cycle. In the presence of a mature follicle the cervix is contracted and edematous and appears open on vaginal examination. During the luteal phase, it becomes dry and the cervical os is usually covered by a flap of the last two cervical rings. During pregnancy the cervix becomes very tight and in the advanced stages the cervix is pulled forward and downward beyond the pelvic brim. The normal size and position of the cervix is regained within the first two weeks following parturition.

1.1.5. Vagina

The vagina is some 25 - 30 cm in length and is lined with many longitudinal folds (Fig. 6 A). The anterior vagina and the vestibulum are separated by a strong band of tissue (vestibulum sphincter muscle) and the hymen. This structure is very tight in nulliparous or young animals and can make manual examination of the vaginal cavity very difficult.

1.1.6. Vulva

The vulva opens directly below the anus and measures 6 - 7 cm in length (Fig. 7A). During the follicular phase edema of the vulva can be present but it is very discrete, however, during the last week prepartum it becomes much more relaxed and edematous. The clitoris is very small and there is no distinct clitoral fossa. The urethra is also short and the opening of the urinary meatus is small.

1.1.7. Reproductive organs in llama and alpaca

The genital organs of llama and alpaca are similar to that of dromedary and bacterian camels, but it is smaller in size. In llama, the outer lengths and luminal diameters of the left uterine horn, from early fetal stages to adulthood, were greater than the right uterine horn. The special anatomical disposition of the uterine septum appeared to restrict the diameter of the lumen of the right horn. No microscopic differences were detected between horns. Additionally, uterine glandular development was found in fetal genital organs at an early age (from CR length 34.5 cm and onward) and no differences were found either between left and right uterine horns or between fetal and adult reproductive organs.

1.2. Reproductive Organs of Mares

The reproductive organs of mare are usually lie in the pelvic and abdominal cavities, while the ovaries hanging in the sublumber region.

1.2.1. Ovary

The ovary is bean-shaped (Figs. 1B, 2B) and is commonly described as having cranial and caudal poles, a lateral and medial surface and dorsal and ventral margins. The dorsal margin is attached by the mesovarium to the body wall. The shape and size of each ovary is variable, dependent mainly on follicular content. There are large variations in shape, size and consistency occurs in normal mares. Anestrous size ranges from 4.1 cm x 2.2 cm x 2 cm to 8.2 cm x 4.1 cm x 4.2 cm; tends to be

larger in older and larger mares. It is suspended in the cranio-lateral part of the broad ligament (the mesovarium). The broad ligament between the ovary and tip of uterine horn is the tuba membrane (free margin of mesosalpinx). The ovary is covered by an extension of the broad ligament (serosa) except at the ovulation fossa, which is a marked depression on its medial surface.

Follicles do not normally protrude above the margin on the ovarian substance unless they are larger than 2.5 cm in diameter. Luteal structures normally protrude above the margin of the ovary for four or five days after ovulation (Fig. 2B), but not during the later luteal phase. During anestrus, ovaries are small and contain small follicles (1–1.5 cm in diameter) and no luteal tissue. Mares in temperate areas and during spring transition, their ovaries tend to be very large and contain many variable-sized follicles (up to five or six follicles, each of 3–4 cm in diameter) and no luteal tissue. During the ovulatory phase, ovaries will variably contain follicles and luteal tissue. It is not uncommon to find significant-sized follicles protruding above the margin of the ovary in mares that are in the luteal phase – at this stage the corpus luteum does not protrude and cannot be palpated.

1.2.2. Uterine tube

The uterine tubes are tortuous tubes measuring 20–30cm in length when uncoiled. The uterine tube runs within the tubal membrane (Fig. 3B). The uterine tube is divided into three regions: (1) Isthmus –commencing at the oviductal papilla at the utero-tubal junction; (2) Ampulla – the area where fertilization and early embryonic development occurs; (3) Infundibulum – which has distinct fimbriae positioned adjacent to the ovulation fossa, between the proper ligament of the ovary and the tubal membrane.

1.2.3. Uterus

The uterus is T- or Y-shaped in appearance; consisting of a body and two horns (Figs. 1B, 4B). Its position may be changed by the degree of filling of the bladder or intestine. The body runs cranially on the ventral floor of the pelvis and caudal abdomen. The uterus is normally dorsal, dorso-lateral or lateral to the bladder. The uterine body averages 20 cm in length. The horns bifurcate from the cranial end of the body, and run laterally, or dorso-laterally. The horns are an average of 20–25cm in length. The horns are smaller in diameter at their tips. The normal non-pregnant uterus has a potential lumen. The thickness of the uterine walls, and the tone of the myometrium, varies significantly with reproductive state and age. Pregnancy causes gross distortion of the shape of the uterus. The two large broad ligaments suspend the uterus in the abdomen. Each ligament extends from the dorso-caudal border of a uterine horn and the dorso-lateral border of the body to the sublumbar and lateral pelvic wall. The continuous sheets of the broad ligaments are commonly divided into three regions: (1) Mesometrium – supports the uterus; (2) Mesosalpinx – supports the uterine tubes; (3) Mesovarium – supports the ovaries.

1.2.4. Cervix

The cervix is a tubular organ 4 - 10 cm long and 2 - 5 cm wide that protrudes into the cranial vagina. It is the last line of defense between the uterine lumen and external environment. The length, diameter, tone and patency of the cervix vary greatly during different reproductive states. It has many longitudinal folds (Fig. 5B).

1.2.5. Vagina

The vagina is a potentially hollow tube which, when undisturbed, is completely collapsed. Cyclical changes in the appearance of the vaginal mucosa are minimal. Normally, there is little bacterial or other contamination of the vagina. Clinical examination may result in a transient inflammation. Most of the vagina is retroperitoneal. A vulvo-vaginal constriction is found just cranial to the external urethral opening (Fig. 6B). It is probably a partial remnant, but in some maiden mares

complete hymen presents at this junction. In genitally healthy mares this constriction forms a secondary line of defense against aspirated air and fecal material. The vestibule extends from vulval lips to vestibule-vaginal constriction. It has pink to brownish-red mucous membrane. Ventrally houses the clitoris which is surrounded laterally and ventrally by clitoral fossa. The dorsal clitoris is covered by the transverse frenular fold. The dorsal surface of the clitoris contains up to three small cavities, the clitoral sinuses. There is always a central sinus and there may be two lateral sinuses. Clitoral sinuses and fossae contain a variable amount of smegma. Correct identification of the sinuses is important to enable proper bacteriological screening of mares prior to breeding.

1.2.6. Vulva

The vulva lies ventral to the anus and is therefore at risk of fecal contamination. The normal vulva is almost vertical in position, and the vulval lips are opposed (Fig. 7B). The angle of the vulva should be evaluated with respect to the vertical. Its length should be compared with the position of the bony pelvis (ischial tuberosities) which can be palpated by finger pressure on the perineal tissue adjacent to the vulva. The normal vulva is vertical, or no more than 10° from the vertical. More than 75% of the vulval length is normally positioned ventral to the bony pelvis. The vulval lips should be firmly closed. Three distinct vulvo-perineal conformational types are recognized. An increase in the angle of declination and/or a decrease in the length of the vulva below the bony pelvis result in increased likelihood of fecal contamination and of an ineffective vulval seal. Presence of air alone will result in a low-grade vestibulitis/vaginitis. With or without bacterial involvement, this may seriously impair fertility. The perineal tissue surrounds the vulva and includes tissue ventral to the tail and around the anus. This region is frequently injured at foaling. The normal anus is dorsal to, and vertically in line with, the vulva. The normal position results in fecal material falling clear of the vulva at defecation. The position of the anus is influenced by the body-condition score of the mare. In thin mares, for example, the anus may be sunken-in, i.e. cranial in position compared with normal.

1.3. Reproductive Organs of Cows

The female genitalia lie in the pelvic cavity and consist of the ovaries, uterine tubes, uterus, cervix, vagina, and vulva (Fig. 1C).

1.3.1. Ovary

The two ovaries lie slightly medially to the tips of the uterine horns to which they are joined directly by parts of the broad ligaments, the ovarian ligament. They are oval in shape and vary in size from about 1.5–5 cm in length and 1–3 cm in diameter, depending on the stage of reproductive cycle (Fig. 2C). Ovarian structure is not static, and the appearance of the surface of the ovary continually changes in cycles of follicle growth and regression or ovulation and corpus luteum growth and regression. The production of ova or eggs, takes place from a group of cells, the oogonia, which develop in cords from the germinal epithelium surrounding the fetal ovary. These cells penetrate the ovarian stroma and gradually differentiate, the larger ones eventually becoming the primary oocytes or egg cells. The neighboring cells surround the oocyte and provide sustenance, the whole structure being known as a primary ovarian follicle. Each female animal has a full complement of primary follicles containing primary oocytes at birth, but the number is far in excess of those required during life. The primary follicles enter phases of growth during the lifetime of the animal, a process possibly under hormonal control, although the precise nature of the stimulus is not understood. In the immature female the ovary consists of a cortex containing primary oocytes, each surrounded by a single layer of supporting cells, and a medulla containing connective tissue, nerves and blood vessels. The growth of follicles is controlled by the neuroendocrine system. During follicular growth, the cells surrounding

the primary oocyte increase in number thus increase the number of layers around the cell. With continued development, a cavity or antrum is formed which becomes filled with follicular fluid. Antral follicles can be seen on the ovarian surface. The outer layers of the follicle are the theca externa and interna. The theca externa contains much fibrous tissue while the internal layer is more cellular and contains many blood vessels. The innermost layer of cells is termed the granulose layer. The oocyte itself is surrounded by a non-cellular membrane, the zona pellucida, and is suspended in the follicular fluid by a clump of cells, the cumulus oophorus.

Following ovulation, the cavity of the ovulated follicle is invaded by cells that are derived from the granulosa and theca interna layers of the follicle. These cells are large and termed lutein cells and they are richly supplied with blood vessels. The structure usually protrudes from the surface of the ovary, is yellow-brown in colour and is known as the corpus luteum (Fig. 2C). This structure persists on the surface of the ovary until a few days before the next ovulation, when it begins to degenerate rapidly, a process known as luteolysis. Alternatively if the animal becomes pregnant then the corpus luteum is maintained for the duration of that pregnancy. The corpus luteum consists of two populations of luteal cells: small (15–20 mm in diameter), originating from the theca interna, and large (25–30 mm), originating from the follicle granulose layer. The small cells account for about 90% of the total population.

1.3.2. Uterine tubes

The uterine tubes or uterine tubes serve to transport ova or unfertilized eggs from the ovary to the uterus and pursue a convoluted course through a section of the broad ligament, the mesosalpinx. Each uterine tube is 20–30cm long and approximately 2–3 mm in diameter and can be considered as consisting of three segments. The narrow isthmus extends from the tip of the uterine horn for about half the length of the uterine tube; the ampulla is a slightly wider section eventually opening into the peritoneal cavity via the funnel-like third portion or infundibulum (Fig 3C). This acts literally as a funnel, which serves to capture ova as they are shed from the ovary at ovulation and to facilitate their passage down the uterine tube.

1.3.3. Uterus

The uterus is a hollow muscular organ consisting of a short body and two relatively long cornua or horns. Thus when straightened the organ is Y-shaped, although in life the uterine horns curve downwards and laterally. The size of the uterus depends on factors such as age and parity, but the uterine body is approximately 5 cm in length and the horns are 20–40cm in length and 1.5–4 cm in external diameter in the non-pregnant cow, tapering at the ovarian extremity (Fig. 1C, 4C). The uterus is suspended in the pelvic cavity by the broad uterine ligaments on either side. These ligaments also carry the blood and nerve supply to the organ. Blood is supplied to the organ by the utero-ovarian and uterine arteries of which the middle uterine artery is the largest. The uterine wall varies from 3–10 mm in thickness and consists of three layers: the inner lining or endometrium; a muscular layer, the myometrium; and the outer ‘serosa’ layer. The endometrium or mucosa consists mainly of glandular epithelium and has approximately 120 specialized raised areas known as ‘caruncles’ (Fig. 4C). These are characteristic of the uterus of ruminants and are the points of attachment of the placenta during pregnancy.

1.3.4. Cervix

Commonly known as the neck of the womb, the cervix forms a barrier between the vagina and uterus. It varies in length from 2 - 3 cm in the heifer to approximately 10 cm in the mature cow and has a very thick fibrous wall. The lumen or cervical canal is convoluted and normally tightly closed

except at parturition, although it also dilates slightly at estrus (Fig. 5C). A plug of thick brownish mucus is usually present in the canal during the luteal phase of the cycle and during pregnancy.

1.3.5. Vagina

The vagina extends anteriorly from the vulva to the cervix and is variable in length depending on whether the animal is pregnant or non-pregnant. The urethra opens into the floor of the vagina approximately 10 cm anterior to the vulva and just posterior to this is a blind pouch, the sub-urethral diverticulum (Fig. 6C).

1.3.6. Vulva

The vulva, or lips, form the exterior opening of the reproductive organs and allow for the entry of the bull's penis (or the AI gun) at service and for the expulsion of the calf at birth (Fig. 7C). In the cow, the vulva also forms the exit point of urine from the body.

1.4. Reproductive organs of buffalo-cows

The female reproductive organs of the buffalo are similar to those of the cow in structure and location (Figs. 1D-7D), although the cervix is less conspicuous and the uterine horns are more coiled. As in cattle, the uterine horns are turgid and coiled and have marked tone during estrus; they are flaccid and lack tone during the diestrous period. The cyclic corpus luteum is reported to be smaller and more difficult to palpate than in cattle. In a study on buffalo ovaries and follicular development the weight of the right and left ovaries as averaging 2.72 g and 2.54 g respectively; the right ovary possessed more developing follicles than the left. This seems reconcilable with data recording the incidence of pregnancy in the right and left uterine horns as 67% and 33**%,** respectively, in Nili-Ravi buffaloes in Pakistan. Studies in India, on the other hand, found little evidence of any size and weight differences between the right and left gonads of the buffalo.

1.5. Reproductive organs of ewes and does

The basic anatomy of the ewe's reproductive organs is similar to that of cattle (Fig. 1E). The ovaries are roughly spherical and about the size of a marble (10 to 15 mm in diameter) (Fig. 2E). The uterine tubes extend towards the ovaries but are not connected to them. Instead they end with a small funnel shaped opening which catches the egg when it is released (Fig. 3E). The uterine tubes provide the site of fertilization and early embryo development before the embryo passes to the uterus. The uterus is a small muscular organ that provides protection and nourishment for the developing embryo (Fig. 1E, 4E). It consists of a body and two uterine horns that are continuous with the uterine tubes. The inner lining of the uterus is made up of many button-like projections known as caruncles, which are the sites of attachment for the placenta ('afterbirth') (Fig. 4E). The transfer of nutrients between the ewe and the developing embryo takes place via the placenta. The development of the embryo and later the fetus continues within the uterus for the duration of pregnancy. The cervix separates the uterus from the vagina. In ewe, it is very tortuous making embryo transfer and artificial insemination difficult (Fig. 5E). During pregnancy it is sealed and protects the embryo and fetus from the external environment. At the time of mating and ovulation it is open, enabling passage of sperm into the uterus. The vagina connects the cervix to the vulva (Fig. 6E). The vulva is the external opening of the genital organs, fleshy and has a small tuft of hair at the ventral commissure (Fig. 7E).

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APPENDIX:Tables and Figures

Table 1: Comparative anatomy of the female reproductive organs in farm animals

	Female camel	Mare	Cow	Buffalo-cow	Ewe and doe
Ovary: Size (Length X Breadth)	3.3 X 2.6 cm	8 X 4 cm	1.5-5 x 1–3 cm	2-4x1.5-2.5 cm	1.5x1.4
Shape	bunch of grape	bean-shaped	ovoid	ovoid	almond
Position	Pelvic/abdominal cavities	Sublumbar	Pelvic/abdominal	Pelvic/abdominal	Pelvic/abdominal
Pre-ovulatory follicle	1.3-2 cm	4-5 cm	1.5-2 cm	1.2-1.8 cm	0.6-0.8 cm
Corpus luteum	1.4-2.1 cm	1.5-2 cm	2-3 cm	1.6-2.5 cm	0.9-1.4 cm
Ovarian bursa	Completely covered the ovary	Less developed	Less developed	Less developed	Less developed
Uterine tube: length	17-28 cm	20–30 cm	20-30 cm	18-25 cm	15 cm
Opening in uterine horn	via a narrow orifice	via a narrow orifice	gradually	gradually	gradually
Uterus: Horns	6–10 cm (right) 8–15 cm (left)	20 – 25 cm	20-40cm	18-34 cm	10-13 cm
Body	4cm	20 cm	5cm	4cm	1.5-2 cm
Shape	T- or Y-shaped	T- or Y-shaped	ram horn-shaped	ram horn-shaped	ram horn-like
Position	Pelvic/abdominal cavities	Pelvic/abdominal	pelvic/abdominal	pelvic/abdominal	Pelvic/abdominal
Endometrium	Folds	Folds	caruncles=80-120	caruncles=100	caruncles: ewe=88 doe=150
Cervix: Length x Width	4-6 x 3.6–6 cm	4–10 x 2–5 cm	6-10x3-5 cm	like cattle	3-5 cm
Mucosa	3-6 muscosal folds	longitudinal folds	3-4 muscular rings		like cattle, highly convoluted in ewes
Canal	dilatable	dilatable	convoluted, closed		
Vagina: Length	25 -30 cm	15-20 cm	18-25cm	Like cattle, but shorter	8-10 cm
Mucosa	many longitudinal folds	many longitudinal folds	smooth		Smooth
Vagina : Vestibulum	separated by strong sphincter	slight constriction	slight constriction		Slight constriction
Suburethral diverticulum	absent	absent	present		Very small
Vulva: Length	6-7 cm	7 cm below anus	10-13 cm	Large and fleshy	3 cm
Position	directly under anus		5 cm below anus		Just below anus
Clitoris	ill-developed	well-developed, round	2-5 cm long		Short
Hair	short	no hair	at ventral commissure		short

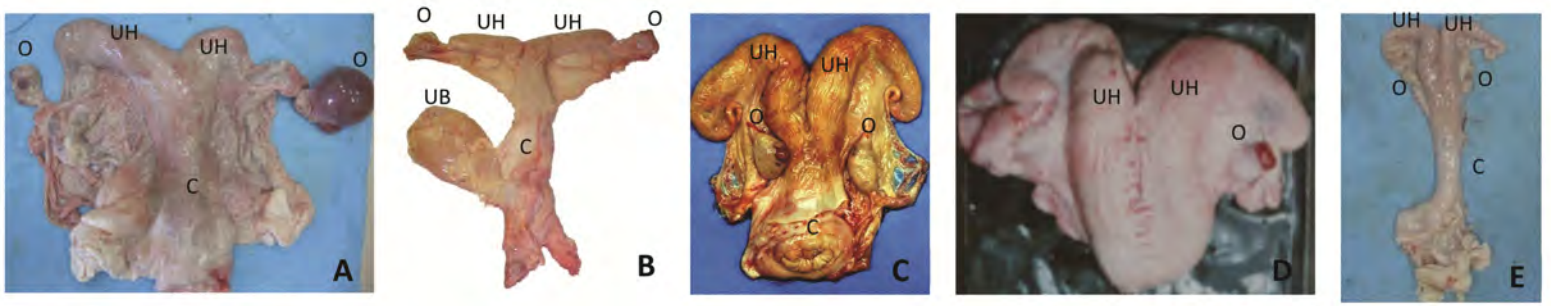

Fig. 1: Reproductive organs of female camel (A), mare (B), cow (C), buffalo-cow (D), and ewe (E). O=ovary, UH=uterine horn, C=cervix, UB=urinary bladder, (Assiut-Egypt, Qassim-KSA, 1988-2013).

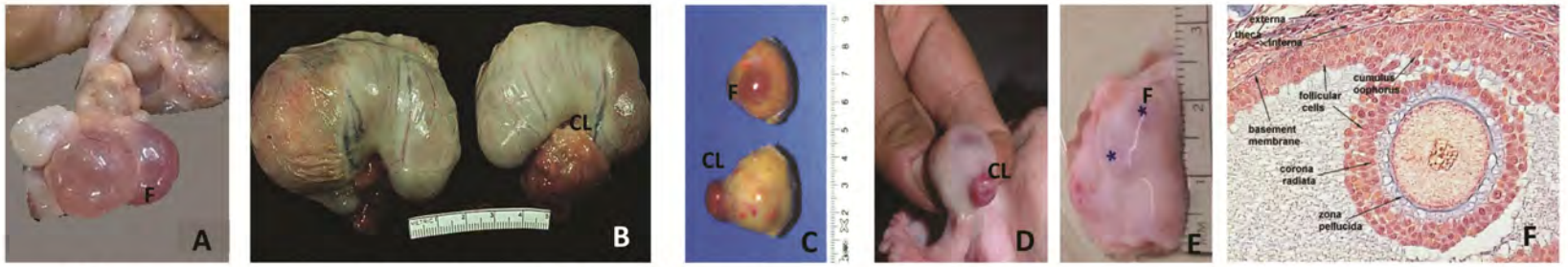

Fig. 2: Ovaries of female camel (A), mare (B), cow (C), buffalo-cow (D), and ewe (E), diagram showing all layers of the follicles and the oocyte (F): F: follicle, CL: corpus luteum, (Assiut-Egypt, Qassim-KSA, 1988-2013).

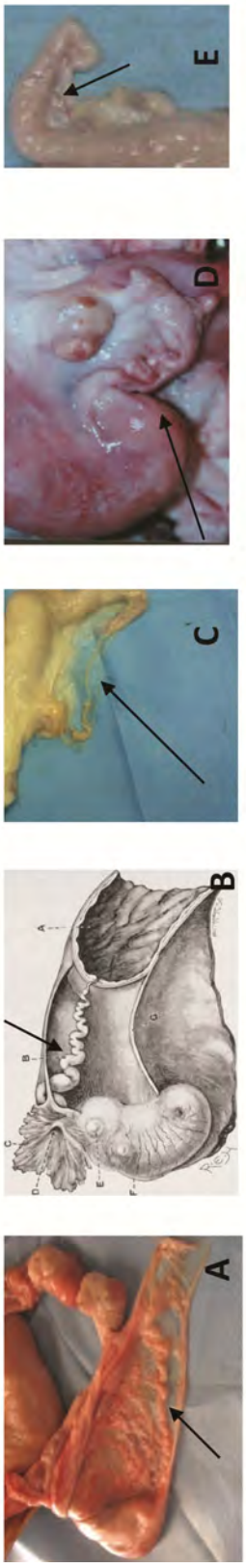

Fig. 3: Uterine tubes (arrow) in female camel (A), mare (B), cow (C), buffalo-cow (D), and ewe (E), (Assiut-Egypt, *Qassim-KSA, 1988-2013; www.uaex.edu).*

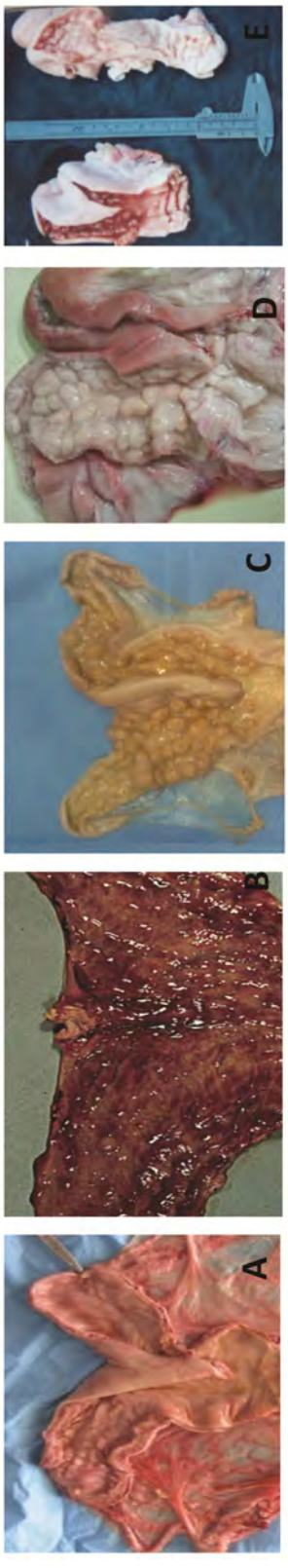

Fig. 4: Endometrium of female camel (A), mare (B), cow (C), buffalo-cow (D), and ewe (left) and doe (right) (E), (Assiut-Egypt, Qassim-KSA, *1988-2013).*

Fig. 5: Cervix of female camel (A), mare (B), cow (C), buffalo-cow (D), ewe (Upper), and doe (lower) (E), (*Assiut-Egypt– Qassim-KSA, 1988-2013).*

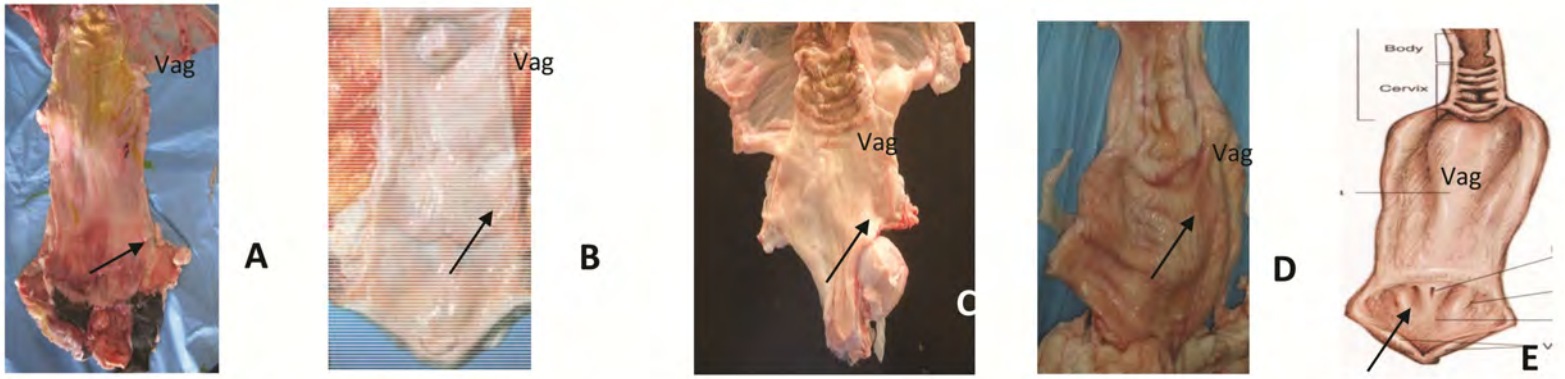

Fig. 6: Vagina (Vag) and vestibulum (arrow) in female camel (A), mare (B), cow (C), buffalo-cow (D), ewe (E), (*Assiut-Egypt– Qassim-KSA, 1988-2013).*

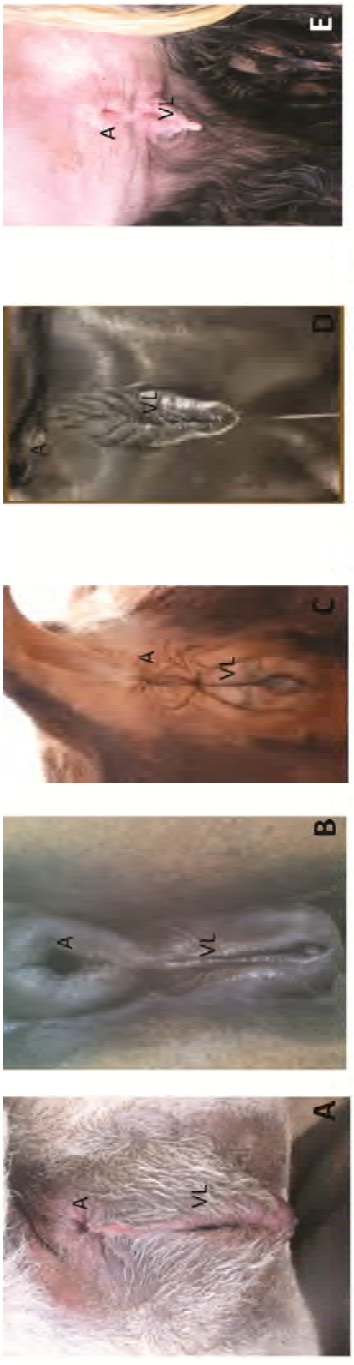

Fig. 7: Vulva (VL) of female camel (A), mare (B), cow (C), buffalo-cow (D), ewe (E). A: anus, (Assiut-Egypt– *Qassim-KSA, 1988-2013*).

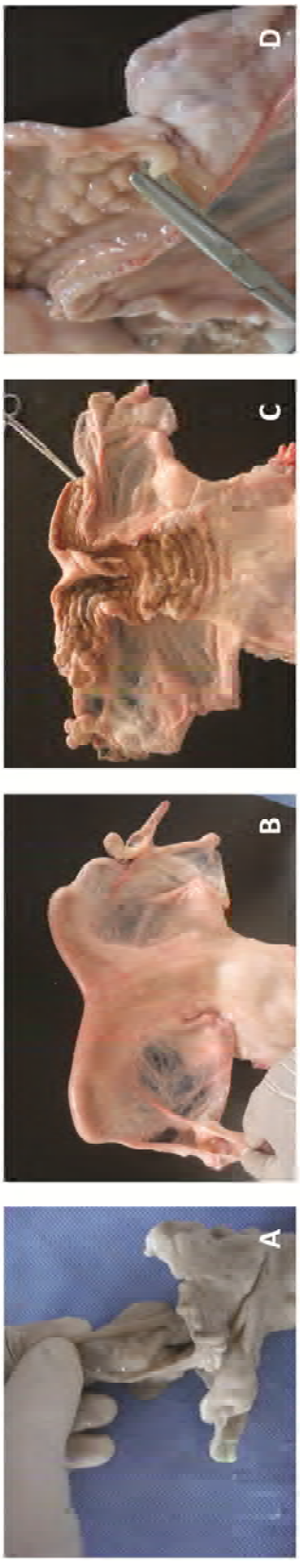

Fig. 8: The ovarian bursa (A, B) compeletly covers the ovary, the fallopin tube opens into the uterine horn with a muscular papilla (C, D) in female camels, (*Qassim-KSA, 2010*).

Chapter 2

The Estrous Cycle

The estrous cycle (also oestrous cycle; derived from Latin oestrus and originally from Greek name meaning sexual desire) comprises the recurring physiologic changes that are induced by reproductive hormones in most mammalian placental females. Estrous cycles start after puberty in sexually mature females and are interrupted by anestrous or pregnancies. Mammals share the same reproductive system, including the regulatory hypothalamic system that releases gonadotropin releasing hormone in pulses, the pituitary that secretes follicle stimulating hormone and luteinizing hormone, and the ovary itself release sex hormones including estrogens and progesterone. Females are generally only sexually active during the estrus phase of their cycle. This is also referred to as being "in heat". The estrous cycle consists of four phases:

Proestrus

One or several follicles of the ovary start to grow. Their number is specific for the species. Typically this phase can last as short as one day or as long as 3 weeks, depending on the species. Under the influence of estrogen the lining of the uterus (endometrium) starts to develop. The female is not yet sexually receptive.

Estrus

Estrus refers to the phase when the female is sexually receptive ("in heat"). Under regulation by gonadotropic hormones, ovarian follicles are maturing and estrogen secretions exert their biggest influence. She then exhibits a sexually receptive behavior, a situation that may be signaled by visible physiologic changes. A signal trait of estrus is the lordosis reflex, in which the animal spontaneously elevates her hindquarters. In some species, the labia are reddened. Ovulation may occur spontaneously in some species

Metestrus

During this phase, the signs of estrogen stimulation subside and the corpus luteum starts to form. This phase typically is brief and may last 1 to 5 days. In some animals bleeding may be noted due to declining estrogen levels.

Diestrus

Diestrus is characterized by the activity of the corpus luteum that produces progesterone. In the absence of pregnancy the diestrus phase (also termed pseudo-pregnancy) terminates with the regression of the corpus luteum. The lining in the uterus is not shed, but will be reorganized for the next cycle.

Anestrus

Anestrus refers to the phase when the sexual cycle rests. This is typically a seasonal event and controlled by light exposure through the pineal gland that releases melatonin. Melatonin may repress stimulation of reproduction in long-day breeders and stimulate reproduction in short-day breeders. Melatonin is thought to act by regulating hypothalamic pulse activity of gonadotropin-releasing hormone. Anestrus is induced by time of year, pregnancy, lactation, significant illness, chronic energy deficit, and possibly age.

The symptoms of estrus phase in different farm animals, the mating behavior, techniques for estrus detection, and synchronization tools are shown in Table (2) and Figs. (9-11).

2.1. Estrous Cycle (follicular waves) in Female Camels

2.1.1. Ovarian dynamics

Camels show increased ovarian activity during cold weather. During this time, the follicles in the ovaries grow, mature and regress if ovulation is not induced by mating. It is therefore more accurate to describe the cyclical ovarian changes in the camel as a follicular wave pattern rather than an estrous cycle. The follicular wave can be alienated into three phases: namely: (i) the growth phase, (ii) the mature phase and (iii) the regression phase. During the growth phase, (lasting on average 10.5 days) a cohort of small follicles develop from a pool of small follicles which grow at a rate of approximately 0.5 - 1 mm/day until they reach about 1 cm in diameter and then one or two follicles become dominant and continue to grow. In about 50% of the cases the dominant follicle grows to a mean maximum diameter of 2.0 cm whilst the others regress, whereas in the other 50% of the cases the dominant follicle continues to grow to a mean maximum diameter of 4.2 cm before it starts to regress, taking on average 18.4 days to reach its maximum diameter. The mature phase includes the time when the follicle has reached maximum size and is ready to ovulate. It lasts on average 7.6 days for mature follicles of 1.5 - 2.5cm and 4.6 days if the follicle grows to as large as 4.0 - 6.4 cm. In the absence of mating, the follicle starts the regression phase taking on average 11.9 days to regress if the follicle measures between 1.5 - 2.5 cm and 15.3 days for the larger follicles. During the regression phase the follicular fluid of the oversized follicles becomes more echogenic owing to the development of free floating echogenic strands, which later become more organized into transecting fibrous bands. These overlarge follicles do not, however, inhibit the growth of other follicles in the same or contralateral ovary, which can mature and ovulate if the appropriate stimulus is applied. In all cases,

new follicles become visible and start to grow before the mature follicle has fully regressed to give an interwave interval of about 18.2 days.

Ovulation in camels is induced i.e. mating with an intact or vasectomized male is necessary to stimulate the luteinizing hormone (LH) surge required for ovulation. However, in dromedaries it can also be induced by gonadotrophin injection, if this is given at the correct time of the follicular wave cycle. The incidence of ovulation is influenced by the size and stage of development of the follicle. In dromedaries ovulation does not occur if the follicle is < 9mm in diameter, but the ovulation rate increases to 85% if the follicle grows to between 1.0 - 1.9 cm in diameter. Then, it decreases sharply if the follicle continues to grow to 2.0 - 2.9 cm (12.5%). No follicles over 3.0 cm in diameter ovulate at all, nor does any follicle in the regression phase. Ovulation in the mated camels is followed in 3 - 4 days by the development of a spherical echogenic corpus luteum (CL) that grows to a mean maximum diameter of 2.2 cm by day 9 after which it starts to regress.

2.1.2. Estrous behavior

Both the dromedary and Bactrian camels are generally regarded as seasonal breeders, however, there seems to be rather conflicting reports as to the beginning and length of the seasonal activity in the dromedary. In some countries, breeding activity seems to be limited to a few months of the year, whereas in others, breeding is continuous, without a real distinction between an anoestrous period and a breeding season. Generally speaking, dromedaries show increased breeding activity during the period of low climatic temperature, rain and better grazing conditions. Although photoperiod is a major factor in stimulating seasonality in many species, well-fed and watered females show ovarian activity throughout the year and the determinant factors of the observed seasonality in conception rates are due to a decrease in libido in the male and an increase in early embryonic death, during the summer months.

Several people have described signs of sexual receptivity or estrous behavior in the dromedary such as: chasing and mounting other females, restlessness, swelling of the vulva, straddling the hind legs and urinating, vaginal mucus discharge, receptivity to the male, and lay down in a sternal position (Fig. 9A). However, all these signs are very variable in duration and intensity and are therefore unreliable for detection of estrus. Sometimes the female can be receptive to the male when there are no follicles in her ovaries or maybe totally indifferent to him when a mature follicle is present!

In llama and alpaca, females lay down in a sternal position when approached by a male or he may mount, push on her withers with his front legs and she quietly lies down after a period of reluctance and submits to mating. Estrous female may seek out the male in pasture situations and lay near a mating couple. In some cases female pacas may mount the male during mating and there may be a long chain of female llamas in the prone position piled on each other on top of a male mating a female. Some nonreceptive females may even assume a sternal posture and submit to mating by a dominant male.

2.1.3. Endocrine control of the estrous cycle

The Hypothalamus secretes GnRH into blood vessels connecting to anterior pituitary. The GnRH acts on specific cells of anterior pituitary to release FSH and LH. FSH secreted into circulation, transported to ovary, and stimulates follicular development. LH secreted into circulation and acts synergistically with FSH to stimulate secretion of Estradiol - 17β by follicle. The concentration of estradiol - 17β in peripheral serum increases initially with the increase in follicle diameter reaching peak values of about 39.0pg/ml when the follicle reaches a diameter of about 1.7 cm. Although the follicle continues to grow to >2.0 cm over succeeding days, the oestradiol - 17β concentrations decline to basal levels again. The Progesterone concentrations remain low for the first 3 - 4 days after ovulation and then rise steadily to a peak of around 2.7 ng/ml on day 8 or 9 before falling sharply again on days 10 - 11 to reach mean values of 0.5 ng/ml by days 11 or 12. The CL has a secretory lifespan of approximately 8.5 days.

2.1.4. Synchronization of follicular wave

Some estrus synchronization protocols have been practiced in camels as trials to develop techniques for fixed time treatments. Exogenous progesterone has been used; however the results are still controversial. It have observed that although the application of PRID (progesterone releasing intravaginal device) (Fig. 11) in camels released adequate amount of progesterone, it failed to produce the desirable suppressive and priming action on the ovaries. It have also reported that PRID alone did not seem to be a satisfactory method of controlling ovarian function in the dromedary camel, premature ovulations induced by PRID prevented the occurrence of well synchronized superovulation. There is the opinion that progestagens priming is not necessary to achieve adequate ovarian response to exogenous gonadotrophic stimulation in the camel. Contrary to the above reports of poor performance with progestagens, other studies have reported very good results using intramuscular administration of progesterone for 14 days. Methods of progesterone administration include daily injections, oral consumption, intra-vaginal devices and subcutaneous implants. Progestagen plus estradiol benzoate treatment facilitated the prediction of emergence of a new follicular wave. Effect of exogenous progesterone on follicular activities has been studied in llama. However, alteration of follicular population to exogenous progesterone treatment has not yet been investigated in dromedary camels.

A study was conducted to synchronize follicle wave emergence prior to superovulation using either GnRH or progestagen treatments, in Bactrian camels. GnRH group camels received 20 μg of the GnRH analogue Buserelin on Days -18 and -4 of the experiment (initiation of superovulation=Day 0). Camels in the progestagen group received two consecutive treatments of progestagens, 7 days apart, on Days -14 and -8 of the experiment. On each occasion, each female received three norgestomet implants and 200mg progesterone (i.m.) and all implants were removed 14 days after the first progestagen treatment coinciding with Day -1 of superovulation. A combination of eCG and FSH was used to induce superovulation and the growth of all subsequent follicles and CLs were monitored daily by ultrasonography. Following the first GnRH injection, mature follicles ovulated within 1-2 days, and a new follicle wave emerged after 3+/-0.77 days. At the time of the second GnRH injection, a mature follicle (15.6+/-0.97 mm) ovulated and a new follicular wave emerged between 1 and 2 days after

GnRH injection. Growing follicles at the time of the first progestagen treatment became either atretic or persistent and a new follicle wave emerged 3-6 days later. At the initiation of superovulation, the diameters of the largest follicle in GnRH and progestagen groups were 7.4+/-0.59 and 20.5+/-2.26 mm, respectively but after superovulation and mating there was no significant differences in the number of unovulated follicles or CLs between groups. In conclusion, two GnRH injections, 14 days apart, may be used to synchronize follicle wave emergence in Bactrian camel.

2.2. Estrous Cycle in Mares

2.2.1. Ovarian dynamics

The mare is a seasonally polyestrous breeder. Ovulation occurs spontaneously at the end of a variable follicular phase. The natural breeding season in the northern hemisphere is May to October. Outside of the breeding season many, but not all, mares become anovulatory. In tropic and subtropics areas, mares have no distinct breeding season and can reproduce all the year. Cycle length is usually 21 days, but it is very variable. Longest cycle length occurs in spring. If cycle length is shorter than 18 days suspect endometritis. *Anestrus u*sually occurs in winter and spring, depending on mare and management system, occasionally occurs in early summer, especially in lactating mares. Small follicles up to 15 mm in ovaries may be found, but there is no functional corpus luteum. It can be diagnosed by palpation of the reproductive organs, ultrasound examination and detection of low plasma progesterone. Transitional period occurs in late winter or early spring, depending on mare and management. Variable follicular activity with many follicles, some may reach ovulatory size before becoming atretic. It is characterized by erratic estrous behavior. Estrous behavior may last more than a month before the first ovulation occurs. *Estrus* is usually lasts 4-7 days, but very variable. It is longest in spring and ends approximately 24 hours (0–48 hours) after ovulation. Split estrus and silent heat may occur. The *interestrus* describes the interval between two successive heats. It is usually 14–16 days in length, but may be longer early in the year, and it may be short if corpus luteum lyses due to endometritis. The *luteal phase* is about 14 and it may be shortened by endometritis. Long luteal phase occurs where corpus luteum is not lysed spontaneously, and it may persist for up to three months (prolonged *diestrus*).

The estrous cycle can be also divided into two phases: (1) Estrus – the period of sexual receptivity; (2) Luteal phase – the period after ovulation during which progesterone is produced by the luteal structures. The luteal phase may also be considered to have two components: the early luteal phase, during formation of the *corpora haemorrhagica* (*metestrus*); and the late luteal phase from approximately day five after ovulation until regression of the corpus luteum on approximately day 15 (*diestrus*). Ovulation occurs approximately 24 hours before the end of behavioral estrus (i.e. estrus is terminated by decreasing concentrations of estrogen and increasing concentrations of progesterone).

2.2.1. Estrus behavior

During estrus the mare is more docile; moves the ears backwards and vocalizes when approached by the stallion; raises the tail, everting the clitoris in a rhythmical manner with frequent urination of cloudy yellow urine (Fig. 9B). Estrous behavior is best displayed when the mare is teased by a stallion (Fig. 10A). Unlike cows, mares do not show overt signs of estrus when maintained with other mares. Mares with foals at foot may not show signs of estrous behaviour.

2.2.3. Endocrine control of estrous cycle

In the non-pregnant mare, endogenous prostaglandin is produced by the endometrium and enters systemic circulation to causes lyses of the corpus luteum. Progesterone concentrations start to decline on approximately day 15. During late diestrus, low-frequency pulses of increased concentrations of GnRH result in release of FSH from the pituitary. Follicle growth is so initiated and these follicles are recruited for ovulation at the subsequent estrus. Estrogen concentrations increase following growth of recruited follicles. Estrogens produce a positive feedback effect on LH which increases in a prolonged peri-ovulatory surge, and then ovulation occurs. Progesterone concentrations increase rapidly after ovulation. FSH concentrations begin to increase again and peak just prior to ovulation.

2.2.4. Manipulation of the cyclic activity

2.2.4.1. Induction of ovulation in cycling mares

Human chorionic gonadotrophin (hCG) is mainly LH-like in effect and intravenous administration of 1500 IU to mares in estrus with a follicle greater than 3.5 cm in diameter will normally result in ovulation within 48 hours.

2.2.4.2. Shortening the luteal phase

Prostaglandin F2α is the treatment of choice. Prostaglandins have two major effects (a) luteolytic and (b) spasmogenic. The spasmogenic effects account for the majority of adverse effects, which can include sweating, increased gastrointestinal motility (colic), dyspnea (especially in mares with chronic lung disease), in coordination and also hyperthermia and hyperglycaemia.

2.2.4.3. Hastening ovulation

The optimal time for mating or insemination of a normal mare is 24–48 hours before ovulation. Gonadotrophin releasing hormone agonists and superagonists, such as deslorelin and buserelin, may be useful. Human chorionic gonadotrophin (hCG) may also be used to hasten ovulation of a large (3.5 cm) follicle.

2.2.4.4. Synchronization of the estrus and ovulation

The estrous cycle of the mare is characterized by a long and variable follicular phase. The time of ovulation within this is variable. Progestagens are most commonly used. Simply, these are given for

a prolonged period so that the only source of progesterone is the exogenous agent. When the exogenous agent is removed, estrus and ovulation ensue. In some cases, ovulation is hastened by administration of hCG or GnRH agonists or superagonists. Progestagens may be administered for 14 days with a prostaglandin being administered during treatment.

2.3. Estrous Cycle in Cows

2.3.1. Ovarian dynamics

Once puberty occurs, estrous cycles generally continue unless pregnancy is established. Estrous cycles typically are 21 days in duration but normally may range from 17 to 25 days. The period of estrus may range from 2 to 50 hours in duration but averages 12 to 18 hours. Ovulation occurs approximately 24 to 30 hours after the onset of estrus. Ovaries of cattle contain two different pools of follicles, the non-growing pool and the growing pool. The non-growing pool contains the primordial follicles, whereas the growing pool contains the primary, secondary and tertiary follicles. Entry of primordial follicles into the growth phase occurs throughout the reproductive life. The primordial follicles continuously leave the arrested pool and undergo the primordial to primary follicle transition. During recruitment of follicles into the growing pool theca cells organize into distinct layers around early developing follicles and establish essential cell-cell interactions with granulosa cells. In cattle the growth of obligatory gonadotropin-dependent follicles occurs in a wave like pattern. Waves of growth can be observed during the pre- and post-pubertal periods. During one interovulatory interval two, three or four waves have been observed. Cycles with three waves were on average 3 to 4 days longer and corpora lutea regressed later than in animals with two waves. Moreover, interval from detection of dominant follicle to ovulation and duration of dominance were shorter in animals with three waves. Greater than 95 % of oestrous cycles are composed of either two or three follicular waves. From the cohort, one follicle is selected for continued growth and becomes dominant. If luteolysis occurs during the growth phase of dominant follicles, final maturation and ovulation occurs. If luteolysis does not occur during the growing and maintenance phase of follicles, the fate is atresia. Recruitment is the entrance of follicles in the growing pool, but also for the processes associated with the entrance of follicles in a wave like growth pattern. FSH is the key hormone for the endocrine initiation of follicular wave occurrence Selection means that the number of growing follicles is brought into line with the species-specific ovulation number. After recruitment fewer and fewer recruited follicles continue in growth until one follicle is selected to become dominant while the remaining members of the recruited follicles become static and undergo atresia via apoptosis. The processes of selection occur under declining FSH concentrations and take 2 to 3 days. Deviation occurs when 2 largest follicles reach 8.3 and 7.8 mm in diameter which is characterized by continued growth of the largest follicle to become the dominant follicle and reduced or terminated growth of the remaining follicles to become subordinate follicles. This was observed at 61.0 h after wave emergence. The LH stimulates the production of estradiol and insulin-like growth factor-1. These intrafollicular factors and perhaps others account for the responsiveness of the largest follicle to the low concentrations of FSH. The smaller follicles have not reached a similar developmental stage and because of their continued and close dependency on FSH become susceptible to the low

concentrations. Follicles are functionally dominant (capable of ovulating after luteal regression) while they are still growing and early during their plateau in growth. Follicles acquire ovulatory capacity at about 10 mm, corresponding to about 1 day after the start of follicular deviation, but they require a greater LH dose to induce ovulation compared with larger follicles. Selection and dominance are accompanied by progressive increases in the ability of theca cells to produce androgen and granulosa cells to aromatize androgen to estradiol. Dominant follicles grow to a much larger size than all the other follicles (from 8.5 mm at the end of selection to 12 – 20 mm). This takes 3 to 4 days. LH pulses are indispensable for follicle development beyond 9 mm in diameter. Follicular dynamics in cattle can be summarized in; (1) follicles grow in a wave-like fashion; (2) periodic surges in circulating FSH are associated with follicular wave emergence; (3) selection of a dominant follicle involves a decline in FSH and acquisition of LH responsiveness; (4) periodic anovulatory follicular waves continue to emerge until occurrence of an LH surge; (5) within species, there is a positive relationship between the duration of the oestrous cycle and the number of follicular waves; (6) progesterone suppresses LH secretion and growth of the dominant follicle; (7) the duration of the interwave interval is a function of follicular dominance, and is negatively correlated with circulating FSH; (8) follicular dominance in all species is more pronounced during the first and last follicular waves of the oestrous cycle and (9) pregnancy, the prepubertal period and seasonal anoestrus are characterized by regular, periodic surges in FSH and emergence of anovulatory follicular waves. Local regulation of ovulation involves the interaction of LH and intrafollicular factors including steroids, prostaglandins, and peptides derived from endothelial cells, leukocytes, fibroblasts, and steroidogenic cells.

2.3.2 Estrous behavior

Standing to be mounted is the most definitive sign of estrus in cows (Fig. 9C). During the period of *standing heat*, cows stand to be mounted by other cows. Cows that move away quickly when a mount is attempted are not in estrus. The average duration of a mount is approximately 2.5 seconds. Secondary signs of estrus include, mucous discharge from the vulva, swelling and reddening of the vulva, bellowing, restlessness, trailing other cows, chin resting, sniffing the genitalia of the other cows, and lip curling. These signs may occur before, during, or after estrus and are not related to the time of ovulation. Such signs should be used as clues that cows are near estrus so that they can be watched more intensely for standing behavior.

2.3.3. Endocrine control of the estrous cycle

With the regression of the corpus luteum, concentrations of progesterone rapidly decline as those of estradiol begin to increase concurrently with the accelerated growth of the preovulatory follicle. As progesterone declines, the baseline concentration of LH increases, the frequency of LH pulses increases from about one pulse every 4 to 6 hours to one pulse every hour, but the amplitude of LH pulses declines. Gonadotropins influence follicular development and subsequent secretion of estradiol. Estradiol appears to exert inhibitory effects on FSH release. A secondary rise in FSH of lower magnitude than that observed during the surge often is reported just before ovulation, or about 24 to 30 hours after the onset of the preovulatory surge. This secondary rise may be a consequence of the loss of follicle-derived inhibin production during the ovulatory process. This increase in FSH

probably is critical to the recruitment of the first wave of antral follicles that becomes visible by intrarectal ultrasonography during early metestrus.

2.3.4. Estrous synchronization

2.3.4.1. Estrus detection aids

A variety of additional aids to estrus detection are available. Traditional aids include pressure-sensitive mount detectors, tailhead markings, and detector animals, monitoring electrical resistance of reproductive organs tissue, pedometry, and electronic pressure-sensitive mount detectors (Fig. 10). A pressure-sensing radiotelemetric estrus detection system that monitors frequency and duration of mounting is commercially available to dairy and beef producers. This externally mounted device, which captures mounting information and transmits it to a receiver interfaced with a computer, has been tested. Results showed the accuracy of estrus detection for this system to be similar to that for visual observation for estrus, with rates of 96% and 94%, respectively. Combining the pedometry component with the mount monitor would make this device a very effective detection aid. The use of several detection aids or monitoring two or more aspects of estrous behavior is superior to a single method for improving the efficiency of estrus detection. The cost, durability, accuracy, functional life, maintenance requirements, access to and interpretation of data, length of time these devices remain attached to the animal, and labor commitment are factors that must be considered in evaluating any new estrus detection technology.

2.3.4.2. Estrous synchronization

Prostaglandin F2α (PGF2α): in one or two doses with 11 to 14 days interval.

Melengestrol acetate (MGA) (0.5mg/day per animal): is fed for a period of 14 days, with PGF2α administered 17 to 19 days after MGA withdrawal.

Select Synch: Gonadotropin-releasing hormone (GnRH) injection, followed in 7 days by an injection of PGF2α.

MGA-GnRH-PGF2α (MGA Select): a) MGA is fed for 14 days, GnRH is administered 10 or 12 days after MGA withdrawal, and PGF2α is administered 7 days after GnRH. b) MGA is fed for 7 days, PGF2α is administered on the last day MGA is fed, GnRH is administered 4 days after the cessation of MGA, and a second injection of PGF2αis administered 11 days after MGA withdrawal.

A controlled internal drug release device (CIDR) (Fig. 11A): It has been developed for the intravaginal release of progesterone and has been proved to be effective for inducing and synchronizing estrus in heifers and cows. The CIDR inserts are placed into the vagina with a lubricated applicator, after disinfection of the vulva. The device has a flexible polyester tail that protrudes from the vulva and is easily removed by pulling the polyester tail. Although mild vaginitis is common with use of CIDR inserts, fertility is not compromised. The retention rate for CIDR inserts is approximately 95%. If the retention rate is considerably less than 95%, the devices may have been inserted incorrectly, or other animals may be pulling out the CIDR insert by biting on the polyester

tails. In the latter case, the problem can be remedied by trimming the polyester tails. The CIDR treatment includes insertion of a CIDR device on day 0, injection of PGF2α on day 6, and CIDR insert removal on day 7. Cows and heifers typically are observed in estrus 24 to 72 hours after CIDR insert removal, and they commonly are inseminated after detection of estrus. PRD is working like CIDR.

CoSynch protocol: Efforts to develop a more effective timed insemination protocol in beef cows have recently focused on synchronizing follicular waves by injecting GnRH, followed 7 days later with an injection of PGF2α and then a second GnRH injection plus insemination 48 hours after the PGF2α injection (*COSynch protocol*). This protocol permits the synchronization of ovulation in cows but at present it has not proved to be as effective in heifers. In cows that have a dominant (≥10mm) follicle at random stages of their estrous cycle, an injection of GnRH will induce ovulation and subsequent formation of luteal tissue. A new follicular wave is initiated in heifers or cows within 2 to 3 days of GnRH-induced ovulation of a dominant follicle, synchronizing the development of a new dominant follicle. Luteal tissue that forms after GnRH administration is capable of undergoing PGF2α-induced regression 6 or 7 days following the first GnRH injection. A limitation to GnRH-PGF2α protocols (including CO-Synch) is that approximately 5% to 15% of the cows are detected in estrus on or before the day of PGF2α injection, so a smaller proportion of females will conceive to the timed insemination 48 hours later.

Ovsynch program: Synchronization of ovulation using timed injections of prostaglandin F2α and gonadotropin releasing hormone (GnRH) (*Ovsynch program*). The objectives of the original Ovsynch research were to (1) hormonally control the onset of a new follicular wave, (2) control the lifespan of the spontaneous and induced CL, and (3) control the time of ovulation of the dominant follicle (DF). Three hormonal injections are needed to accomplish these three objectives. The first GnRH injection causes ovulation, or luteinization, if a functional DF was present in the ovary. Subsequently, if ovulation occurs, a new follicular wave emerges approximately 1.5 to 2 days later. GnRH does not cause ovulation if the stage of follicle development is in the first 3 days of a spontaneous follicular wave. The newly induced wave, or a spontaneous wave if ovulation does not occur, is allowed to develop, with selection and dominance of a DF during the following 7 days. At that time, PGF2α was administered to induce luteolysis, thus allowing for further growth and maturation of the DF. Then, 48 hours later, a second GnRH injection induces a preovulatory luteinizing hormone (LH) surge that triggers ovulation approximately 28 hours after treatment with GnRH. Cows treated with Ovsynch demonstrated overall conception rate similar to those obtained in cows that had been bred to detected estrus (37% versus 39%). AI at 16 hours after the final GnRH injection, when administered 48 hours after PGF2α administration, results in the highest conception rate, compared with AI at 0, 8, 24, and 32 hours after the final GnRH dose.

2.4. Estrous Cycle in Buffalo-Cows

2.4.1. Ovarian dynamics

Buffalo has estrous cycles with 1, 2 or 3 follicular waves; that 2-wave cycles are the most common; and that the number of waves in a cycle is associated with the luteal phase and with estrous cycle length. The first wave begin at 1.00, 1.16 and 1.10 days in buffalo with 1, 2 and 3 waves, respectively (ovulation = day 0). The second wave appears at 10.83 and 9.30 days for the 2 and 3 wave cycle animals, respectively. The third wave starts at 16.80 day. Structural persistence of the first dominant follicle is longer in the 2- than 3-wave cycles (20.67 vs. 17.90 days). The duration of the growth and static phases of the first dominant follicle differs between the 2 and 3 wave cycles, whereas there are no differences in linear growth rates (cm/d). Two and three wave cycles differs with respect to the maximum diameter of both the first dominant follicle (1.51 vs. 1.33 cm) and the ovulatory follicles (1.55 vs. 1.34 cm). No relationship is found between dominant follicle development and the presence of either a CL or a previous dominant follicle in either ovary. Two and three wave cycles also differs with respect to the mean length of intervals between ovulation (22.27 vs. 24.50 days) and the mean length of luteal phases (10.40 vs. 12.66 days).

In swamp buffalo, estrus occurs throughout the year. The estrous cycles corresponding to single ovarian cycles ranges from 11 to 38 days with a mode interval of 20 days, averages 21.5 days. Percentage of the cycles within a range of a mean (17-26 days) was 79.2%, whereas that of cycles shorter or longer than the expected range is 9.4% and 11.4%, respectively. Estrus takes place regardless of the time of day and lasts 9 to 27 hr (19.9+/-4.4 hr). Ovulation occurs 6 to 21 hr (13.9 hr) after the end of estrus, with a mode interval of 12 hr. There are no significant seasonal variations in the estrus characteristics between the two models.

2.4.2. Estrous behavior

In a study on Murrah buffaloes, swollen vulva was the best indicator of estrus followed by excitement and chasing by bull (90%). The first and longest duration of estrus signs was swollen vulva, which was seen up to 21.6±1.1 h after onset of estrus. In another study on the same buffalo breed, the vulval discharge of clear mucus in varying quantities in a recumbent animal, even in silent heat, was the most reliable single sign of heat in buffalo heifers. Acceptance of male is the most accurate indicator (Fig. 9D). Of the other signs observed, wall walking (segregation) and bellowing confirmed estrus in 83.69% and 80.43%, respectively, while placid response of the animal to the placing of palm of hand on the rump and response to light massage of vulval lips were seen in 86.95% and 83.69%, respectively. Based upon the degree of manifestation of estrous symptoms, intensity of estrus was divided into four categories. Four daily observations of the animal for important symptoms of heat a few days before the expected estrus could make estrous detection a sure success. In Egyptian buffaloes, the estrus symptoms in buffaloes were less intense than in cattle and there was no homosexual activity. Proestrus occurred in 43% of the cases with an average duration of 21.20 hr. whilst metestrus occurred in 67% of the cases with an average duration of 19.20 hr. The average duration of oestrus was 28.47 hr. The onset and cessation of oestrus were either abrupt or gradual. The

phenomenon ‘split oestrus’ was observed intervening normal cycles. This was not characteristic of the individuals.

2.4.3. Endocrine control of the estrous cycle

Plasma progesterone concentrations are lowest (0.30 ng/ml) during the peri-estrous phase and increase through the early luteal phase to a maximum concentration (1.94 ng/ml) during the mid-luteal phase. Circulating plasma inhibin and estradiol concentrations are lowest (0.31 and 11.04 ng/ml) during the mid-luteal phase; increase through the late luteal phase to maximum concentrations (0.44 and 22.48 ng/ml) during the peri-estrous phase. Plasma FSH concentrations are lowest during the early luteal phase and increase through the mid-luteal phase to a maximum concentration during the peri-estrous phase. Peripheral prolactin concentrations are lowest during the late luteal phase and increase to a maximum concentration during the peri-estrous phase which then decline during the early luteal phase. Peripheral plasma cortisol concentrations decrease from 2.68 ng/ml during the early luteal phase to 1.43 ng/ml during the mid-luteal phase then increase to 2.06±0.17 ng/ml during the late luteal phase. Plasma T3 concentrations decrease from the late luteal phase to the peri-estrous phase which then increased during the early luteal phase. T4 concentrations increase from the late luteal phase to the peri-estrous phase then decrease during the early luteal phase.

2.4.4. Estrus synchronization

Responses of two types of PGF2α were studied in buffalo-cows. Ninety-three percent of the buffaloes came in estrus with a mean interval to the onset of estrus of 73 hours following injection of PGF2α. The sign of estrus and behavioral changes revealed that 56.25% of the experimental animals showed mucus discharge followed by frequent urination (37.5%), mounting (25%), and swelling of the vulva (18.75%).

A study was conducted by the authors to compare two estrus synchronization protocols in buffaloes. Animals were divided into two groups: Group A received 100 μg GnRH on Day 0, 375 μg PGF2α on Day 7 and 100 μg GnRH on Day 9 (Ovsynch); Group B received an intravaginal drug release device (PRID®) containing 1.55 g progesterone and a capsule with 10 mg estradiol benzoate for 10 days and were treated with a luteolytic dose of PGF2α and 1000 IU PMSG at the time of PRID® withdrawal. Animals were inseminated twice 18 and 42 h after the second injection of GnRH (Group A) and 60 and 84 h after PGF2α and PMSG injections (Group B). The findings indicated that treatment with PRID® can induce ovulation in non-cyclic buffalo cows. However, synchronization of estrus with Ovsynch resulted in a higher pregnancy rate compared with synchronization with PRID®, particularly in cyclic buffalo.

Another study by the authors aimed to evaluate ovarian dynamics and progesterone concentrations in cyclic (CYC) and non-cyclic (NCY) buffalo-cows during Ovsynch program. All buffalo-cows received GnRH on day 0, $PGF_2\alpha$ on day 7, and GnRH on day 9, and AI 14 h later. Ovarian structures were monitored by ultrasound and milk samples were collected for progesterone (P4) analysis. The first GnRH resulted in ovulation in CYC (90%) and NCY (62.5%) cows. By day 7, almost all cows had large follicle and lutein tissue. Luteolytic responses to $PGF_2\alpha$ were 80 and 87.5%

for CYC and NCY cows, respectively. Following second GnRH, ovulation occurred in 80% of CYC and 100% of NCY cows. Ovulation began earlier (12 h following second GnRH) and extended for longer (36 h) in NCY cows, when compared to CYC cows (36 and 12 h, respectively). The mean P4 levels increased from days 0 through 7 in CYC and NCY cows and levels were higher in CYC group. Conception rates were 60 and 37.5% in CYC and NYC cows, respectively. Early and asynchronous ovulation and luteal sub-function seemed to be a problem in NCY cows. Inseminating NCY cows twice, at 0 and 24 h of the second GnRH was recommended.

2.5. Estrous Cycle in Ewes and Does

2.5.1. Follicle dynamics

In sheep in subtropics, development of individual follicles and corpora lutea (CL) in were studied by the author and his co-workers in Ossimi ewe lambs at different seasons of the year. Ovarian follicles ≥2 mm and corpora lutea were counted and measured. Three (65%) and two (35%) follicular waves were detected per estrous cycle. None of the characteristics of the large follicles was affected by season. Follicles ≥2 mm in diameter were significantly higher in winter. The CL developed slowly in autumn. Serum P4 level was higher in autumn. Double ovulation was observed only in autumn. The average cycle length is 17 days. Ovulation normally occurs toward the end of estrus. Typical ovulation times for the ewe are about 24 to 27 hours from the beginning of estrus.

In one study on 6 goats, the mean interovulatory interval f was 20.8d. The incidence of goats with 4, 3, and 2 follicular waves was 3, 1 and 2 respectively; follicular waves emerged on Days 0.5, 7.2, 10.7 and 13.7for wave 1, 2, 3 and the ovulatory wave, respectively. The largest follicle of Wave 2 was smaller (4.9 mm) than the largest follicles of Wave 3 (6.2 mm) and of the ovulatory wave (7.0 mm), and tended to be smaller than the largest follicle of Wave 1 (6.3 mm). Interval between emergence of wave 1 and wave 2 was longer than interval between emergence of wave 2 and Wave 3 (7.3 d vs. 4.0 d), and between wave 3 and the ovulatory wave (3.8 d). Two days before ovulation, the diameter of the ovulatory follicle was larger than the first subordinate follicle. Serum E2 concentrations increased from the day of ovulation (2 7 pg/mL) to Day 2 (7.6 pg/mL), associated with the early-mid growing phase of the largest follicle of wave 1, and then decreased to basal levels on Day 5 and peaked again (16.5 pg/mL) 2 d before ovulation. The CL was detected ultrasonically on Day 3 post ovulation and attained a mean maximum diameter of 13.5 mm between Days 8 and 1.

2.5.2. Estrous behavior

Sheep and goats are seasonally polyestrous and short-day breeders, meaning they will cycle regularly starting with the shortening days of fall. For goats, the most natural time is usually from late July through December, but tropical breeds may cycle throughout the year. The signs of estrus in ewes are less noticeable than they are in does. A doe in heat is restless, bleats and urinates frequently, wags her tail rapidly, and accept the male (Fig. 9E). She may also experience loss of appetite and rub against other goats in the herd. Other signs include redness and swelling around the vulva, which may have a thin mucous discharge. The signs of estrus in the ewe are not as easily detected when the ewe

cannot hear, smell, or see the ram. Sheep and goats do not mount, or stand to be mounted, as often as cattle do.

2.5.3. Endocrine control of the estrous cycle

The temporal relationships between LH, estradiol and progesterone in peripheral serum of the ewe were characterized throughout the estrous cycle. Between successive preovulatory LH surges, serum concentrations of LH fluctuated markedly in a manner indicative of pulsatile discharges. Mean serum LH and progesterone concentrations were inversely related, LH being highest during the early and late luteal phases of the estrous cycle and lowest in the mid-luteal phase. A progressive, 5-fold increase in serum LH concentrations occurred between the onset of the precipitous fall in circulating progesterone attendant to luteolysis and the initiation of the preovulatory LH surge. Two major increments in circulating estradiol were observed in each cycle, both occurring when serum LH concentrations were relatively high. One estradiol increment occurred during the early luteal phase, the other during the 2–3 days prior to onset of the preovulatory LH surge. The latter estradiol increment thus accompanied the progressive, 5- fold increase in circulating LH which precedes the LH surge. These observations are inconsistent with the view that tonic LH secretion in the ewe is solely a function of a negative feedback action of estradiol. Rather, the temporal relationships between circulating hormones, in conjunction with recent findings that progesterone can inhibit tonic LH secretion in the ewe, lead to the conclusion that progesterone plays a major role in the regulation of tonic LH secretion during the estrous cycle of sheep.

Progesterone, estrogen, LH, FSH and PRL variations in plasma were measured in goats. Mean progesterone level was 0.0-0.8 ng/ml in estrus and 1-4 ng/ml in diestrus. Estrogen levels ranged from 10 to 20 pg/ml in diestrus; around estrus, a peak of 26.9 +/- 3.18 pg/ml was observed. LH values were 0.5-3.0 ng/ml with a preovulatory peak (40.7 +/- 10.12 ng/ml) 8 to 24 h after the onset of estrus. Except for a peak (14.0 +/- 3.09 ng/ml) coincident with LH surge, plasma FSH level ranged between 2 and 4 ng/ml. PRL level averaged 2-5 ng/ml in diestrus but high concentrations of this hormone were seen around estrus.

2.5.4. Estrus synchronization

Estrus synchronization in sheep and goats is achieved by control of the luteal phase of the estrous cycle, either by providing exogenous progesterone or by inducing premature luteolysis. The latter approach is not applicable during seasonal anestrus, whereas exogenous progesterone in combination with gonadotropin can be used to induce and synchronize estrus in anovular does and ewes.

A study was aimed at finding the best synchronization method in early anestrous season in ewes. The study was performed on Kalkuhi ewes. The ewes were randomly divided into 3 equal groups. In group A intra vaginal sponge containing 60 mg of MPA was left in the vagina for 14 days. In group B intra vaginal CIDR (Fig. 11B) was left for 12 days. Immediately after removal of the Sponge and CIDR, PMSG hormone at the doses of 500 IU was administered intramuscularly in both groups. Group C received two injections of prostaglandin (Lutalyse 3 cc) 11 days apart. After the

treatment, 5 fertile rams were released among each group for 4 days. This practice was then repeated after 15 days to detect the estrus ewes. The percentage of gestation of the ewes in all groups was then recorded to compare the effect of the treatments. The results showed that while %45 of the ewes in group A and %35 in group B were gestated, the statistic for group C was %70. The study suggests that in transitional period from estrus to anestrous, synchronization with PG yields better results than other methods in Kalkuhi ewes.

Also, a study performed by the author was aimed to evaluate three regimes for estrus and ovulation synchronization in Farafra ewes in the subtropics. During autumn, ewes were assigned to (i) controlled internal drug releasing (CIDR)-eCG group, treated with CIDR for 12 days and eCG at insert withdrawal; (ii) PGF2α-PGF2α group, treated with two PGF2α injections at 11 days interval; and (iii) GnRH-PGF2α-GnRH group, treated with GnRH, followed 5 days later with PGF2α and 24 h later with a second GnRH. Oestrus-mating detection was carried out at 4 h intervals starting on day 0 [the day of CIDR withdrawal (CIDR-eCG group), the day of second PGF2α treatment (PGF2α-PGF2α group) and the day of PGF2α treatment (GnRH-PGF2α-GnRH group). The obtained results showed that, oestrus expression, ovulation and conception were greater in CIDR-eCG and PGF2α-PGF2α groups than in GnRH-PGF2α-GnRH group. All ewes of PGF2α-PGF2α group presented, on day of second PGF2α injection with mature CL (P4>2.0 ng/ml), compared to 42.9% in GnRH-PGF2α-GnRH group. The peak of oestrus occurred 32-52, 48-60 and 28-96 h after the end of treatment in CIDR-eCG, PGF2α-PGF2α and GnRH-PGF2α-GnRH groups, respectively. Ovulation started 48 h after treatment in all groups and extended for 24, 36 and 48 h for CIDR-eCG, PGF2α-PGF2α and GnRH-PGF2α-GnRH groups, respectively. Results demonstrated that oestrus and ovulation synchronization could be efficiently achieved in Farafra ewes using either CIDR-eCG or PGF2α-PGF2α regimes; however, the GnRH-PGF2α-GnRH treatment induced a more spread oestrus and ovulation that may make the protocol inadequate for timed artificial insemination.

The efficiency of medroxyprogesterone acetate (MAP) and fluorogestone acetate (FGA) sponges (Fig. 11D) with or without PGF2α (cloprostenol) for synchronizing estrous in non-lactating does was investigated during the natural breeding season. Does were treated for 11 days with 60 mg MAP or 40 mg FGA sponges. All does also receive intramuscular injections of 500 IU PMSG. Cervical artificial insemination (AI) with diluted fresh semen was performed at a fixed time (36 and 48 h) following progestagen withdrawal. These results indicate that, the use of MAP/PMSG and FGA/PMSG intravaginal progestagen treatments are equally efficient in synchronizing estrous in non-lactating hair goats during the natural breeding season.

Using PMSG or equine chorionic gonadotropin (eCG) is similar to using FSH and LH. These hormones promote follicular growth and development and can be applied intramuscularly 48 hours before or at the moment of withdrawing the CIDR on day 8 or 18. After withdrawing the CIDR apparatus, apply 300 IU to 500 IU of PMSG intramuscularly. Artificial insemination can be conducted by appointment 54 hours after removing CIDR. The pregnancy rate is 50 percent using this method.

Prostaglandin PGF2α has also been used effectively to synchronize estrus in goats. PGF2α promotes luteolysis or corpus luteum regression and lowers progesterone levels. Progesterone acts on

the pituitary gland, inhibiting FSH and LH secretions. A doe responds to prostaglandin treatment if there is an active CL in the ovary. This method is effective only if applied in cycling does, and it can be applied following the introduction of the teaser or male effect. Prostaglandins can be found on the market as the natural product dinoprost tromethamine or as the synthetic product cloprostenol. A method of synchronization consists of two injections of cloprostenol or a synthetic prostaglandin (PGF2α) compound given at 62.5, 125, or 250 ml intramuscularly in 10- to 14-day intervals. However, this method is only effective in synchronizing does during the breeding season when they are cycling and have an active corpus luteum. Cloprostenol can be administered between day 5 and day 16 of spontaneous estrous cycle; the dose is 16mg, 8 mg twice intramuscularly, 11 days apart. Artificial insemination must be performed 50 hours after the last injection of prostaglandin. These protocols can be used only in cycling does or during the cyclicity period, offering over 90 percent efficacy.

A study assessed the efficacy of an Ovsynch protocol (vs. the classical cronolone containing vaginal sponge + eCG treatment) to generate fixed-time insemination in goats during the breeding season. Each regimen was applied to 24 Boer goat does. Onset and duration of estrus were determined with an aproned male and follicular development was monitored by ultrasonography. Ovulation and quality of the corpora lutea were established from progesterone concentrations. In 10–11 goats per group, LH concentrations were determined throughout the preovulatory period. Does were inseminated at pre-determined times (16 h after the second GnRH injection and 43 h after sponge removal). Estrus was identified in 96% of the Ovsynch-treated goats (at 49 h after prostaglandin injection) and in 100% of the goats synchronized with sponges (at 37 h after sponge removal). Low progesterone concentrations at the time of AI were observed in 21/24 and 24/24 goats synchronized by Ovsynch and sponges, respectively. Synchronization of the LH surge was tighter following Ovsynch compared to sponge treatment. Kidding rates (at 58 and 46% in the Ovsynch and sponge groups, respectively) and prolificacy (at 1.86 and 1.83 in the Ovsynch- and sponge-treated goats) were similar for both groups, as were the number of ovulations (2.9 and 3.3) and the proportion of does with premature corpus luteum regression (29 and 17%). When excluding does with premature luteal regression and those with low progesterone levels when receiving prostaglandins, kidding rate reached 87.5% (14/16) after Ovsynch. During the breeding season, the Ovsynch protocol may thus be an useful alternative to the sponge–eCG treatment.

The buck or "male effect" is an effective management practice that requires a minimal amount of labor and cost. The pheromone of the buck will stimulate the hypothalamus of the does to segregate GnRH that, in turn, stimulates the anterior pituitary gland to secret FSH and LH. Ovulation in a proportion of does can occur within 24 to 72 hours after male exposure. The first male-induced ovulations in noncycling does may result in short cycles of 5 days. Doe fertility rate is increased by 15 percent after the second and third estrus following buck exposure. To achieve the buck effect, does must remain isolated from males for at least 30 days. Does that have been in constant contact with the males during anestrus will take longer to begin cycling when reintroduced to the buck. However, heat followed by ovulation can vary according to body condition and the health status of the herd.

Controlling photoperiod is another management alternative that does not require hormonal treatment; however, this method requires adequate facilities and labor. It is a well-applied practice in dairy goat management of induction of heat and ovulation. This method consists of controlling exposure to daylight in an effort to mimic short-day periods.

Does and bucks are confined to separate pens where they encounter alternating light exposure consisting of a long photoperiod followed by short-day darkness, such as 16 hours of light exposure and 8 hours of darkness. Then, there is a short period of light exposure followed by a long period of light exposure, such as 8 hours of light to 16 hours of darkness. This protocol should be applied in 90-day intervals. It will result in LH pulses to stimulate ovarian activity. Cyclicity will begin after 40 to 70 days for mature does and after 70 days for yearlings. This method has a 90 percent success rate. Does do not require hormonal treatments, but they require adequate facilities.

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APPENDIX: Tables and Figures

Table 2: Estrous cycle in farm animals

	Female camels	Mares	Cows	Buffalo-cows	Ewe and doe
Breeding season	Cool months	spring and early summer (long days)*	all over the year	all over the year	late summer and fall (short days)**
Estrous cycle length	24 days	22 days	21 days	21 days	Ewe: 16.5 days Doe: 20 days
Estrus phase (heat)	5 days	5 days	18 hours	18 hours	Ewe: 36 hours Doe: 48 hours
Estrus behavior	sternal position, seeking male, urination, moisted vulva, switching tail	raising tail, urination, rhythmic contraction of the clitoris, accept the male	clear viscous discharge from vulva, mounting other animals, stand to be mounted	bellowing, moving, accept the male	seeking and accept the male, moisted vulva, frequent urination stance
Type of ovulation	induced	spontaneous	spontaneous	spontaneous	spontaneous
Time of ovulation	32 hours after mating	38 hours before end of the estrus	12 hours after end of the estrus	12 hours after end of the estrus	toward the end of estrus
Mating time	first day of estrus	2^{nd} and 4^{th} days of estrus	12 hours after estrus beginning	12 hours after estrus beginning	first day of estrus

* In subtropics: all over the year

** In subtropics: all over the year

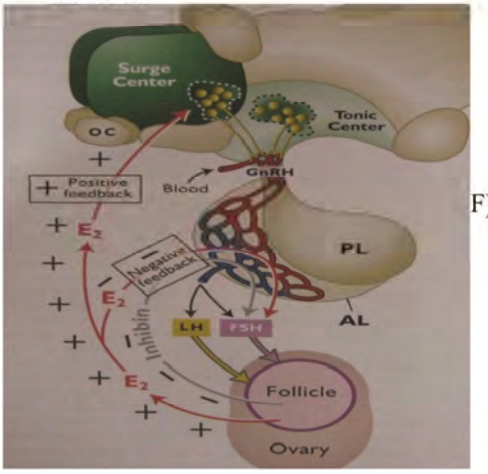
A) Camel: submissive behavior toward the male

B) Mare: raising tail, frequent urination

C) Cow: stand to be mounted by other cows or bulls

D) Buffalo: stand to be mounted

E) Doe,ewe: search and accepte the male



F) hormonal control of the estrous cycle (*Senger, 2003*)

Fig. 9: Estrus symptoms, mating behavior and hormonal control of the estrous cycle in farm animals (*Berlin-Germany, Qassim-KSA, 1998-2010).*

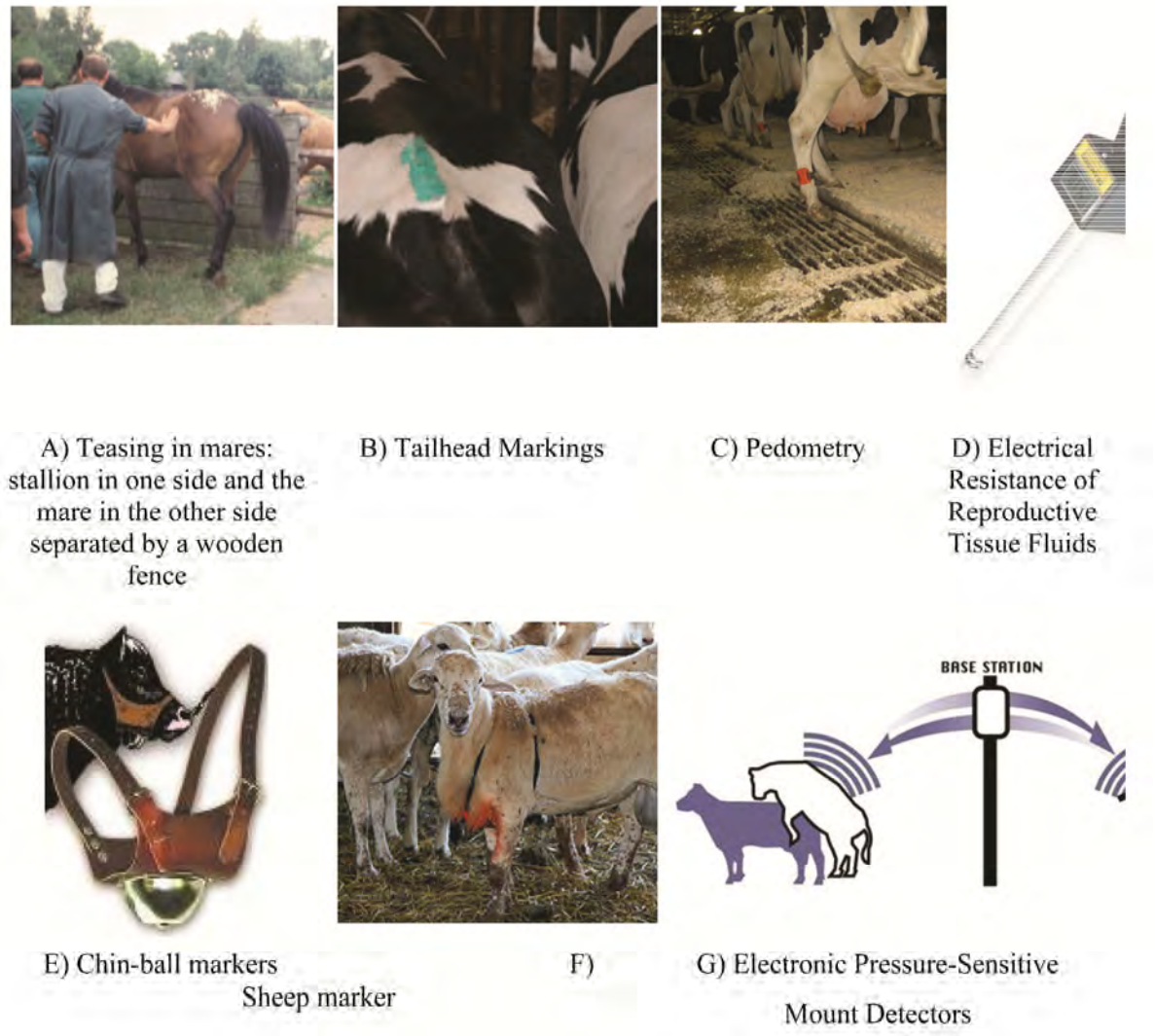


Fig. 10: Techniques for detecting estrus in farm animals (Berlin-Germany 1995-2000; www.uaex.edu).

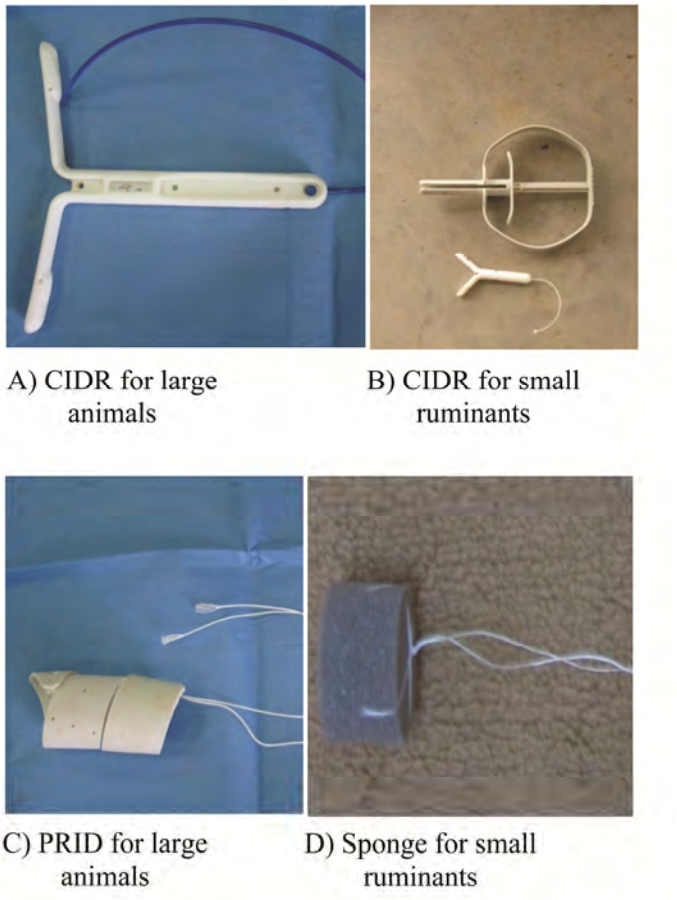


Fig. 11: Synchronization tools (Assiut-Egypt, 2005).

Chapter 3

Reproductive Efficiency and Management of the Female Animals

Reproduction is a vital factor in determining the efficiency of animal production. Good reproductive performance is essential to efficient management and production as a whole, although specific reproductive targets may depend to an extent on local conditions and on individual farm systems and targets.

Puberty is defined as the onset of the first estrus associated with a potentially fertile ovulation that is followed by a luteal phase of normal duration. Puberty represents only the onset of sexual maturation, which actually is not achieved until the female reaches the maturity characteristic of her breed. Puberty occurs when gonadotropins (FSH and LH) are produced at high levels enough to initiate follicle growth, oocyte maturation, and ovulation. Follicle growth can be detected several months before puberty. As puberty approaches, pulse-like releases of gonadotropins become more frequent and of higher amplitude. When they approach adult levels, they stimulate resumption of oocyte maturation and ovulation occurs. A number of environmental factors have a pronounced effect on age at puberty. In general, any factor which slows growth rate, thus preventing expression of full genetic potential, will delay puberty. High environmental temperature delays puberty.

Comparison of the reproductive performance and management systems in farm animals are shown in Table (3) and Fig. (12).

3.1. Reproductive Efficiency and Management in Female Camels

3.1.1. Reproductive efficiency

Sexual activity in dromedaries and bactrians has been reported to start as early as 2 - 3 years of age but usually they are not bred until they are 4 years old. There are several factors that can influence the age of the onset of puberty, such as nutrition, season of birth and breed of camel. The most important factors being the nutrition and adequate growth, as these seem to influence the onset of ovarian activity and chances of conceiving and carrying the pregnancy to term. If females are bred before they reach 70% of their adult body weight they run a greater risk of abortion. Induction of puberty at as early as 1.5 - 2.5 years has been attempted by several people using single or multiple injections of equine Chorionic Gonadotrophin (eCG: 1000 - 7000 i.u.; Folligon) but even though the majority of females responded to such treatment, the incidence of early embryonic death was high. In

addition, the calves born to eCG treated females lagged behind in their growth and development up to 14 months of age. Thus trying to advance the age of first conception using this treatment cannot be recommended.

Reproductive efficiency in the female camels under natural condition is low when compared with other domesticated species. For example, the calving rate of approximately 40% for 30 herds has been reported in Tunisia and a mortality rate of 17% between birth and 1 yr of age. This is probably due to the relatively short breeding season, a longer prepubertal period, a long gestation period of 13 months, a prolonged (8–10 months) period of lactation-related anestrus leading to a long inter-calving interval, and the lack of use of efficient assisted reproductive techniques such as embryo transfer and artificial insemination. The low fertility rate in camels may be due to non-developing follicles, embryonic mortality and abnormal anatomical features of genital organs of the she-camel, failure of females to ovulate when mating and poor semen quality. Improvement of management conditions is very likely to increase the fertility rate above 50% in camels. Eighty percent of animals have a calving interval of at least 2 years and 73 do not rebreed within 12 months of calving. Calving rate averaged only 40% in a Soviet camel ranch, 41% in Egyptian camels, 9.82 - 60% (average 39.2 during 1959 to 1984 and 35.38 and 51.47% during 1985 and 1986, respectively) in Indian Bikaneri camel and 39.1% in dromedary camel in Libya. The wide year to year variations were due to inconsistent management in the different years. Unplanned breeding, malnutrition and poor management practices result in low calving rate. The maximum calving was during January, followed by February March, December, April, May and November, respectively. Ratios of male to females during the breeding season were stated to be 1 male to 5-7 females and 1 male to 50-80 females. A male camel can serve up to 50 females in a season and 70 females when it is very well fed. Moreover, one camel stallion can breed three females per day at the peak of the breeding season depending on levels of management and health. The recommended ratio is 1 male to 20-25 females. Keeping extra males is desirable to provide genetic diversity and to check inbreeding and for wider and efficient selection Herd growth in camels is affected by late age at first calving, limited breeding season and opportunity, prolonged calving interval, low plane of nutrition, poor management practices, diseases and frequent prenatal losses Improvement of the reproductive efficiency of the camel could be very acceptable if a calf per each she-camel is produced every 2 years. Maintaining adequate nutritional level, advancing puberty, achieving conception outside the breeding season and shortening the days open, may be beneficial, in that respect. The use of A.I. may realize that level of the reproductive efficiency.

3.1.2. Reproductive management

The introduction of controlled breeding programs is important but several problems have to be considered. For example, estrous behavior is very vague and difficult to interpret, as it does not often relate to follicular development in the ovaries. In addition, all camelids are induced ovulators that normally ovulate only in response to mating, so alternative methods of inducing ovulation, such as injecting gonadotrophic hormones, have been investigated. The use of embryo transfer is becoming increasingly important but involves the necessity to superovulate the donors and synchronize the recipients so that they ovulate preferably 24 h after the donor. Superovulation can be achieved using

exogenous gonadotrophins, although there is a high incidence of follicle luteinization before mating, of overstimulated ovaries and non-responsive females. The development of AI in camels is complicated by the difficulty of collecting semen and the gelatinous nature of the semen produced. However, diluting semen in Green Buffer and inseminating a minimum of 300 x $10^{(6)}$ live spermatozoa has given encouraging results. The ability to control the follicular cycle of camels is leading to an improvement in reproductive efficiency. The ability of camels to cope with food shortage is the result of a long evolutionary process in natural conditions where food ability seasonally fluctuates.

In northern Kenya, using the Progeny History surveying technique, data were collected from 471, 287 and 416 adult Rendille, Gabra and Somali female camels including data on 1506, 789 and 1206 parturitions, respectively. Age at first calving was 58.4 months, 63.0±1.1 months and 68.4±1.3 months for the Somali, Rendille and Gabra camels, respectively. The mean calving interval was similar for all three populations with 27.3 for Rendille camels, 28.0 for Gabra and 28.4 months for Somali camels. The annual calving rate varied between 33% and 46% in the Somali, 19% and 44% in the Gabra and 8% and 86% in the Rendille camel population. Calf mortality rate averaged 25%, 22% and 27% in Rendille, Gabra and Somali camel calves, respectively, and showed highest variation between the years in the Rendille system (5% to 60%).

Number of matings during the follicular cycle and data on conception rate were collected and analyzed in camels (Bikaneri, Jaisalmeri and Kachchi breeds) managed under semi-intensive system. Ovarian activity during breeding and non-breeding season was examined ultrasonographically. It was revealed that follicular growth and regression is a gradual and sequential process in absence of ovulation. Apart from breeding season, follicular growth was also observed in 50 percent of the camels during non-breeding season. An improvement of about 10 to 15 percent could be observed in conception rate when given 2 matings at interval of 72 hours as compared to single mating and 2 matings at an interval of 24 to 48 hours.

In Libya, like other North Africa Arab Countries, the camels are concentrated mainly at the arid lands, where they are well adapted to the hard environment. A total number of 3120 heads of camels within 38 herds were included in this study. The management of camels in Libya is mainly of three types: continuous and temporary traveling, or permanent stay. Size of the herd differs greatly, with a mean number of about 82 herds ranging between 63-100 heads. Mature she-camel occupies between 50-60% of the total herd number, while the mature male camel, which are used for reproduction, does not exceed 2% of the total herd number. The rest include young male and female camels. Although reproductive activities in camels are mostly seen during October till April, there are evidences that some camels could breed naturally all around the year. Mean gestation period in the reproductive female camels was 375 days, ranging between 365-390 days affected by different factors. Sex-ratio of the new- born calves was about equal (almost 49.5 % males and 50.5 % females). Female camels have good reproductive ability which reached about 72%, while the productive ability was about 67%. Some she-camels suffered from reproductive diseases affecting their reproductive and productive abilities. Such abnormalities include abortion (5%), dystocia (6%), retained placenta (about 8%), and death of newborn calves (5%) within the first two weeks postpartum.

3.2. Reproductive Efficiency and Management in Mares

3.2.1. Reproductive efficiency

Puberty commonly occurs at two years of age, but some mares ovulate as yearlings in late summer, especially if born early in the year. Factors that influence puberty are thought to include: photoperiod – a progressive increased day length is most effective at inducing puberty; good body-condition score/nutrition anecdotally result in earlier puberty; pheromones from other mares in estrus may enhance the onset of puberty; training and/or the administration of anabolic agents may delay the onset of puberty.

In a study for the author and his co-workers on Arabian mares in Saudi Arabia, the age at first mating was 3 years; the horsemen organized themselves to mate their mares either in winter or allover year; most of the horsemen used natural mating (76.9%), some used both natural mating and artificial insemination AI (16%), while few used only AI (8%); the median estrus duration was 7 days and the estrus interval was 20 days; the overall pregnancy rate was 87 % (range 40-100%); the median number of cycles/pregnancy was 1.39 (range 1.1-2.36); the median pregnancy duration was 335 days (range 320-360 days); the median interval from foaling to mating was 9 days (range 9-90 days); and the median weaning time was 6 months (range 5-8 months). In King Abdul Aziz Arabian Horses Center located at Dirab the median age at first foaling was 4 years (range 4-7 years); the reproductive life extended - in some mares up to 17 years; the maximum number of foals given by a mare was 11; the median number of foals/mares' age was 0.39 (range 0.15-0.69); and the median foaling interval was 15.9 months (range 11-34 months).

Horses have always been selected by man for breeding on the basis of their performance or conformation, i.e. they have never been selected for fertility. Pregnancy rates at any one heat may vary from 40–70% in large breeds of horse; this value is generally higher for ponies. Some apparently normal mares require mating at up to four heats to become pregnant; others fail to conceive until the next season. Overall pregnancy rates at the end of the season vary between 50% and 90%, and this depends upon: fertility of the stallion; fertility of the mares; value of the horses involved, i.e. intensive veterinary management of mares, where cost warrants this, results in better fertility, and very expensive stallions do not usually attract mares which have low fertility. Pregnancy loss, after confirmed conception, is about 15%; this figure is lower for ponies.

3.2.2. Reproductive management

Good studs tease mares regularly and individually; this involves the employment of sufficient trained staff. Mare owners who want to transport their mare to the stallion when in heat must be aware that the mare may have fooled them, and that recently travelled mares may not be relaxed enough to accept service. Many mares which arrive at stud said by their owners to be in heat are not. Mares fail to exhibit heat for many reasons; this may be a management fault, but is more often a problem of the mare. The length of time for which a mare fails to show heat before veterinary advice is sought depends on the policy of the stud and the attitude of the owner. Such a decision should involve consideration of: the cost of veterinary treatment; the cost of keeping a mare at stud; the stage of the

breeding season. Veterinary attention to a brood mare may be of benefit to three different factions: (1) The stud in general: e.g. swabbing for venereal diseases and postmortem examination of aborted fetuses and dead foals, i.e. identification of specific diseases, allows measures to be taken to prevent spread; (2) The mare owner: e.g. examination and treatment of mares not seen in heat, pregnancy diagnosis, treatment of mares with endometritis and examination of mares which repeatedly fail to hold to service; (3) The stud owner: e.g. examination to ascertain the time of ovulation so that the mare only needs to be mated on a limited number of occasions; if the stallion has a lot of mares booked to him (i.e. is popular) this procedure is necessary to facilitate organization of an efficient mating program. Mistakes made by mare owners which contribute to poor fertility include: presenting the mare for only one day when she is thought to be in season; taking the mare home after mating and assuming that failure to observe subsequent heat is a reliable indicator of pregnancy; not allowing the stud owner to request reasonable veterinary attention to the mare; presenting a mare to stud late in the breeding season on a whim or due to a leg or other injury which precludes other use of the mare.

There are many ways in which mares may be managed upon a stud. The method chosen depends upon the veterinary surgeon's experience and stud manager's ability.

Extensive management: Veterinary surgeon visits weekly or twice weekly; mares teased daily. Mares should be mated every 48 hours until the end of estrus.

Intensive management: Veterinary surgeon visits stud daily or every other day; teasing uncommon; mares examined repeatedly and cycles manipulated; mares mated once during each cycle at an appropriate time in relation to ovulation. The intensive management system has several advantages and disadvantages: Improved knowledge of stage of the cycle; more accurate timing of mating; improved rate of conception to first service; mare gets pregnant sooner and spends less time at stud; more efficient use of the stallion; more efficient detection of abnormalities so that they can be dealt with rapidly; academically more interesting for the veterinary surgeon; increased cost, but this may be similar if the mare is managed extensively and does not get pregnant at the first mating.

3.3. Reproductive Efficiency and Management in Cows

3.3.1. Reproductive efficiency

Prepubertal heifers may have at least one anovulatory estrus that precedes their first normal cycle (13% to 22% of heifers at an average of 3 months before puberty), often called a nonpubertal estrus. Heifers fed the high-energy and protein diets were 15 d younger at conception and 14 d younger at calving than heifers fed the conventional diet.

Heifers born early in the calving seasons are usually heavier at weaning and reach puberty earlier than heifers born late in the calving season. Heifers must reach puberty by 13-14 months of age to calve as two-year-olds. Puberty is influenced by age, weight and breed. The onset of puberty, as well as of nonpubertal estrus, is influenced by several factors, including age, genotype, season, body weight, nutrition, and social rearing environment. Heifers should have their first calf by 2 years of age.

In some countries, especially in the tropics, much of the cattle production could be described as multi-purpose, with cows being used to provide milk, meat, clothing, fertilizer, fuel, draft power and sometimes for status or as a form of currency. However, for the most part, cattle production may be divided into two sectors: dairy production and beef production. In much of mainland Europe and the developing countries, the same cattle are used as a source of both beef and milk and therefore have a 'dual purpose'. The aim of breeding is to utilize individual parents of high quality in both characteristics. By contrast, in countries such as Australia, New Zealand, the USA, and Canada the functions of beef and milk production have been separated and selective improvement of livestock is directed towards a single characteristic. The situation in the UK is intermediate between these two extremes in that the beef and dairy industries are interdependent.

A cow is only likely to produce a single calf per year. In the dairy herd, the goal of ever-increasing milk yields is often pursued to the exclusion of other factors. However, a cow will only begin to lactate effectively after calving and milk yield will eventually cease unless she calves again. Rearing and maintenance costs are high in intensive systems, so that any delay beyond two years to first calving, and any increase in calving interval beyond the optimum, is likely to cause a significant reduction in income. Calves are important both as heifer replacements and for the production of beef. The reproductive process is thus of vital importance.

Factors affecting the calving to conception interval

In order to achieve a 365-day calving interval the calving to conception interval should not be more than 80–85 days. For the purpose of recording reproductive performance on the farm the calving to conception interval is often subdivided into two components: the calving to first service interval and the first service to conception interval. The calving to first service interval depends on (1) the re-establishment of ovarian cycles after calving, and (2) the occurrence and detection of estrus.

3.3.2. Reproductive Management

In order to maximize efficiency, and specifically reproductive efficiency, in a dairy or beef herd the farmer should (1) develop a series of targets, (2) manage the herd and individual cows so as to best achieve those targets efficiently and (3) evaluate performance on a regular, ongoing basis, in order to correct problems before they have a very serious impact on the attainment of the targets. The targets that need to be aimed for fall into a number of overall categories: culling and replacement policy; the rearing of heifer replacements and calving patterns.

Culling and replacement policy

The policy for culling cows from the herd can have dramatic effects on the reproductive performance of the herd. Culling may be planned, i.e., deliberate policy, or unplanned, e.g., following disease or injury. To maximize genetic progress, a high proportion of cows should be culled each year and replaced with higher genetic merit heifers. On the other hand, cows need to remain in the herd for five or more lactations to maximize economic returns, and heifers in their first, second and even third lactations will not be yielding up to their genetic potential. Thus, culling rates should never exceed 25%, and culling rates greater than 20% are not advisable in dairy herds.

Management to achieve reproductive targets

As a basic rule of thumb, cows should be served as soon as possible after day 60 postpartum. If the estrus detection rate is approximately 75% and there are no disease problems, then an average 365-day interval should be achieved with little variation. There is often a temptation to start inseminating earlier, in case a subsequent estrus is not detected. Conception rates are likely to be lower, and subsequent death of the conceptus will be more likely, especially in high-yielding cows. In herds served too soon, the result may be that the average calving interval is close to 365 but that the variation is rather large. If the calving to conception interval is much less than 85 days (i.e., a 365-day calving interval), the presence of the fetus has a tendency to depress milk yield when it should be near maximum and at the end of lactation the cow will have to be dried off sooner than normal, while milk production is still high. It is also likely that the dry period would be too short to allow proper regeneration of the udder so that production will be low in the ensuing lactation. Conception later than 85 days after calving will lead to calving intervals greater than 365 days, with serious economic consequences for many cows. High-yielding cows could continue to lose body condition until at least 100 days after calving. Energy balance, calculated from daily measures of feed intake and milk output, returned to positive values at days 72, 75 and 95 in lactations 1, 2 and 3, respectively. It is thus clear that some cows not only achieve good economic performance at calving intervals of more than 365 days, but also will have difficulty conceiving and maintaining their pregnancy if they are served on or before 85 days postpartum.

Recording of reproductive performance

The keeping of accurate records is necessary for good herd reproductive management. Recording systems have been developed to a more sophisticated state for dairy cows, but records are equally important to maximize fertility in the beef herd. A number of types of recording systems are available. They include: a simple diary; event recording sheet; display board; computer systems; individual cow cards. The information that should be recorded (input) should include at a minimum: cow identity; calving date; estrus (bulling) dates; earliest date for service; service date; bull used for service; result of pregnancy diagnosis and other veterinary treatments. The recording system should be capable of quickly providing the following information (output): cows ready for service; cows not served by target date; cows ready for pregnancy diagnosis; cows pregnant; cows to be dried off (dairy); cows due to calve. Whilst a number of commercial computer recording and analysis packages are now available, the simple recording of information on the farm is still necessary. In addition to the output data described above these commercial packages usually provide periodic summaries of herd fertility status.

Days Open

Better described as the calving-to-conception interval, in the past this was the most widely used parameter to assess “overall” reproductive performance in a herd. Calculated on an annual basis, days open has significant momentum and lag and is distorted by exclusions, culling, “do not breed” cows, and assumed outcomes. Different record systems deal with these issues in different ways. Nonetheless, it is a readily available and understandable parameter and so remains in widespread use.

In addition to the difficulties inherent in calculating average days open, depending too much on this single number may mask serious reproductive inefficiency from a wide distribution of individual cow performance. Suffice it to say, although some veterinarians will continue to use average days open as a historical assessment of reproductive efficiency, prudent practitioners will do so with care.

Calving Interval

Although maintaining a short calving interval is the conceptual goal of reproductive management, the parameter itself is fraught with problems. As noted earlier, calculating an actual calving interval requires that a cow has calved twice. The parameter has severe momentum and excludes first-lactation animals and culled cows. It is the weakest monitor of a herd's reproductive performance, and should not be used.

3.4. Reproductive efficiency and Management in Buffalo-Cows

3.4.1. Reproductive efficiency

The average age of first estrus, first conception and first calving were 406, 647 and 963 days respectively. The body weight at first estrus and first conception were 198 and 319 kg, respectively. The number of services/conception ranged from 1 to 7 with an average of 4.25. The number of silent heats/female ranged from 1 to 4 with an average of 1.65. The period elapsing from first estrus to first conception ranged from 52 to 438 days. Before conception, there was a period of anoestrus which ranged from 115 to 314 days; this was probably due to weak estrus symptoms. The live weights at 28, 84, 140, 196, 252 and 308 days were correlated with the age and live weight at first estrus as well as the live weight at first conception.

Puberty is significantly affected by: breed; season; climate; feeding systems and growth rate. Buffalo may be considered to be seasonally polyestrous; the female *River buffalo* is active from July until the end of February. The peak of first matings occurs during autumn and winter. Swamp buffalo *shows* continuous cyclicity throughout the year, but a crop-associated seasonal pattern is observed.

The calving interval of buffaloes varies between 400 and 600 days, although longer calving intervals are no exception. Seasonal, nutritional and managerial factors play an important role. The first ovulation in river buffaloes does not generally occur before 55 days post partum, but may be delayed up to day 90 post partum when a suckling calf is present. The first estrus is detected after 130 days post partum in suckled cows, but may be delayed much longer depending on nutritional and climate conditions.

Reproductive efficiency is hampered in female buffalo by the late attainment of puberty, seasonality of calving, long postpartum anoestrus and subsequent calving interval. Proper estrus management allows for more efficient planning of milk production, especially in larger herds and facilitates the use of artificial insemination. Essentially similar basic approaches are being used in buffaloes as there are in cattle. All of the pharmacological systems for estrus management used

nowadays in buffaloes have been adapted from the systems used in cattle through empirical approach and are supported with increasing number of controlled works reported in the literature. The same products are being used in buffaloes as in cattle although few of them have indication for buffalo specifically stated in their user leaflets.

3.4.2. Reproductive management

A study was designed to determine the possibility to improve the reproductive performance of buffalo cows through the continuous exposure to bull with grazing and free-stall housing management. Sixty-four Egyptian multiparous buffalo cows raised under two different management systems in two farms were used in this study. The cows in the first farm (management system 1, MS1) were loose—housed in a free-stall yard, grazed for 4 h per day, suckled their calves for 2–3 months and were continuously exposed to a fertile bull. The cows in the second farm (management system 2, MS2) were confined in an open-fronted tie-stall shed, not grazed, suckled their calves for only 7 days and were exposed to a fertile bull twice per day (30 min per session). All the cows were fed a diet of green berseem (*Trifolium alexandrinum*), rice straw and concentrate to meet their maintenance and production requirements. The cows during both the treatments were milked twice per day after weaning. The cows in both groups were between the second and the sixth parity, weighed 450–480 kg and had average daily milk yields of 5.0–6.0 kg. In each farm, cows were visually checked twice daily at 07:00 and 17:00 h for the signs of estrus and animals proved standing heat were naturally mated. Rectal palpation was used to monitor uterine involution and for pregnancy diagnosis. Blood was sampled twice per week from 7 to 150 days post-partum for serum progesterone assay. The results revealed that post-partum intervals to each of first ovulation, first oestrus, conception and next parturition were significantly shorter in MS1 group than in MS2 group. In the meantime, MS1 increased the conception and calving rates by 21 and 25%, respectively compared to MS2. Percentages of post-partum cyclic animals and animals exhibiting ovulatory oestrus were greater in MS1 group than in MS2 group. However, the percentage of animals cycling before day 60 post-partum was significantly lower in MS1 group than in MS2 group (13% versus 28%). By day 120 post-partum, only 63% of the buffaloes were cycling in MS2 group versus 94% in MS1 group. Percentage of silent ovulation was insignificantly higher in MS2 group (34%) than in MS1 group (25%). However, the percentage of false oestrus was higher in MS1 group than in MS2 group (16% versus 3%). In addition, percentage of short ovulatory cycles (15–17 days) was greater in MS1 group than in MS2 group, whereas percentage of long ovulatory cycle (25–28 days) was higher in MS2 group than in MS1 group. It was concluded that continuous exposure of buffalo cows to a fertile bull with grazing management under free-stall housing system enhances resumption of post-partum ovarian activity and improves conception and calving rates.

3.5. Reproductive Efficiency and Management in Ewes and Does

3.5.1. Reproductive efficiency

Most breeds of sheep will reach puberty when they are 40 to 50% of their mature weight, but breeding is recommended until they are about 65% of their mature weight. Age at puberty is affected by both genetic and environmental factors. Genetic factors can be seen by comparing of breeds within a species. Average age at puberty is 5 to 7 months for does and 6 to 9 months for ewes.

Sheep are short-day or fall breeders. Their breeding season is initiated as the ratio of daylight to darkness decreases and when increasing day length reaches a ratio of nearly equal daylight and darkness. For most breeds the season falls between the autumnal equinox and spring equinox. However, sheep may extend breeding seasons if environmental conditions (Nutrition and climate) are favorable. Moreover, sheep in tropics and subtropics area are not seasonal. Quiet ovulations (ovulation without behavioral estrus) occur more frequently at the beginning and at the end of the breeding season. Introduction of rams into the flock during the transition from anestrus to estrus (from late summer to early fall) will result in a high degree of synchrony in first mating, with estrus peaking 15 to 20 days after introduction of the male. Most subtropical breeds reach puberty late, possibly due to low growth rates.

Although the genetic quality of a sheep and goat herd is important, reproductive traits in sheep have low heritability. Trying to improve the reproductive efficiency of a sheep and goat herd by genetic selection is slow and difficult. Reproductive traits are responsive to environmental influences, however, and they respond to careful herd reproductive management. Some important factors for sheep and goat producers that must be considered are age, weather, season, and nutrition.

Puberty, the time of first sexual activity, has a marked effect on lifetime production. Nutrition is a factor influencing the start of puberty. Overfeeding ewe lambs and doelings to get them to a heavy weight quickly, however, does not guarantee that a high percentage will show estrus early. They must also be old enough to cycle. Sheep are more susceptible than goats to high temperatures and humidity. Stress caused by high environmental temperatures can seriously affect fertility, embryo survival, and fetal development. High humidity increases the risk of heat stress at any air temperature. A rise in body temperature is what actually causes reproductive problems. Increased body temperatures occur most commonly from high environmental temperatures but can also be the result of disease, fever, or any other factor that increases body temperature for an extended time. The most critical period for conception and embryo survival in the ewe and doe is the first 21 to 30 days after breeding. As with ewes and does, fertility in rams and bucks is also affected by temperature and humidity. Heat stress created by environmental conditions or fever caused by diseases that significantly elevate body temperature for an extended time can interfere with sperm production and development, thus affecting semen quality. The fertility of rams and bucks can be affected within days of exposure to extreme heat, and it can take at least 6 to 10 weeks before sperm quality returns to normal. Shearing the ewe flock and rams 2 to 4 weeks before breeding can help reduce heat stress. Rams can also be turned out only at night during hot weather to minimize heat stress. Extremely cold temperatures can be harmful too, especially during bitterly cold weather with high wind and wind chill. The scrotum and even the

testicles can freeze in such extreme conditions. Periods of sickness, as stress factors, can also slow or stop sperm production temporarily.

The nutritional status of a herd is the most important factor influencing reproduction. It is also the factor over which the producer has the most control by either increasing or reducing nutrient consumption. The body condition of a ewe or doe strongly affects the following: the time at which puberty starts; the conception rate at first estrus in ewe lambs and doelings; the length of the postpartum interval; the health and vigor of newborn lambs and kids Body condition or changes in body condition before and during the breeding season affect reproductive performance in terms of services per conception, lambing and kidding intervals, and the percentages of open ewes and does. Ewes and does should be in good body condition at lambing and kidding and should maintain good body condition during the breeding season.

3.5.2. Reproductive management

Pasture breeding and artificial insemination are the two methods of breeding used in sheep and goats, with pasture breeding being the most commonly used. The main advantage of pasture breeding is the reduction in required labor. After the rams and bucks are put in with the ewes and does, all that is required is an occasional visit to the pasture to see that the males are actually with the ewes and does and that the females are becoming pregnant. A marking harness can be used with sheep to help determine that the ram is mounting although this does not guarantee that breeding has occurred.

The number of ewes or does that a ram or buck can service under pasture breeding depends on the length of the breeding season, the age of the ram or buck, and the type of housing (pasture, paddock, range, etc.). Yearlings and two-year-olds are still growing and should avoid excessive loss of body weight during the breeding season. A good management practice in this case limits the number of females to 15 to 30. Producers must provide supplemental nutrition to young rams and bucks after their first breeding season to bring their body condition back. Remember, they are still growing. A young ram or buck should generally not be put in a pasture with an older, more experienced ram or buck because the younger males are often intimidated and sometimes injured by the older ones.

The use of artificial insemination allows producers to use superior rams and bucks to dramatically improve lamb and kid performance in the areas of birth weight, weaning weight, and muscling. However, the rewards of artificial insemination depend on sound management.

Alternatives to induce out-of-season estrus and estrus synchronization during the breeding season

A gestation period of 148 days makes it possible for a ewe or doe to give birth more than once a year. But because of the seasonality of anestrus, ewes and does do not cycle after spring kidding and lambing until late summer or early fall resulting in just one lamb or kid crop per year. If the ewe and doe could be induced to come into estrus and breed during this seasonal anestrus period, they could lamb and kid in the breeding season and produce three in two years (8-month interval) or twice a year (6-month interval). A possible breeding scheme would be to breed in January, then again in September. Out-of-season breeding programs help producers attempting to increase the profitability of their operations by increasing the supply of lamb and cabrito to the marketplace on a year-round basis.

Goats generally respond more favorably than sheep to out-of-season breeding. Two methods for inducing out of season estrus are light control (photo period) and the ram or buck effect.

Light Control

Altering the day-length pattern by controlled lighting can be used to induce estrus. The change of day length from long days to short days initiates the estrous cycle in sheep and goats. Rams and bucks as well as ewes and does should be exposed to the same amount of light every day. Exposure of rams and bucks to short days will increase sperm production, mating activity, and semen quality. The amount of light should be reduced gradually over an 8- to 12-week period.

Ram or Buck Effect

When a ram or buck is introduced to a group of females, the ewes and does come into estrus. This effect is known as the ram or buck effect. The male effect works best in breeds that are less seasonal and during the transitional breeding season (July through August) when most ewes and does have not yet begun to cycle but are almost ready. During the nonbreeding season, some females may even be stimulated to ovulate and express estrus. The male effect relies on females and males being totally isolated from each other for at least 1 month. Ewes and does must be far away from rams and bucks so no contact is made by either sight or smell. The initial ovulation will be a nondetectable "silent heat" at 3 to 4 days after the introduction of the ram and buck. Two peaks of estrus activity follow this around days 18 and 25. Ewes that do not conceive may cycle again in 17 days. In does, ovulation occurs 2 to 10 days after introduction of the buck. The male effect works because rams and bucks produce chemical substances called pheromones, the smell of which changes the reproductive physiology of the female and stimulates her to start cycling. The value of the ram or buck effect is the synchronization of estrus activity resulting in large numbers of ewes and does ovulating, conceiving, and birthing in a relatively short period of time. To be effective, it is important to have adequate numbers of young, healthy rams and bucks. Teaser or vasectomized ram and buck can also stimulate the ram or buck effect.

Management factors affecting out-of- season-induced estrus and breeding

The management and care of ewes and does have an impact on the success of out-of-season breeding. They must be in good body condition, preferably gaining body weight at the time of breeding. Flush - a term meaning to provide excess energy - the ewe or doe before and during breeding. The start and duration of flushing depends on the body condition of the animals. Rams and bucks must also be in good body condition. Poor nutrition can decrease testicular size and sperm reserves at a time when the size and reserves are already smaller than during the breeding season. Production of spermatozoa takes 7 to 8 weeks. As a result, supplementary feeding must begin 8 weeks before the start of the breeding season to increase sperm reserves. Seasonal variations with respect to semen production, semen quality, and libido should also be considered. Elevated body temperatures from hot weather can cause temporary infertility. Shear rams 2 months before breeding and be sure that all wool is removed from the scrotum area. Another very important factor is to ensure that adequate ram power is available for out-of-season breeding. Rams are not able to breed as many ewes out of season.

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Appendix: Tables and Figures

Table 3: Reproductive performance of farm animals

	Female camels	Mares	Cows	Buffalo-cows	Ewes and does
Age of puberty	2-3 years	2 years	9 months	13 months	5-7 months
Age of sexual maturity	4-5 years	2-3 years	15 months	18-24 months	9-12 months
Age at first parturition	5-6 years	3-4 years	24 months	36 months	1-1.5 years
First estrus after parturition	3-8 months	9 days	35-45 days	60-120 days	60-90 days
Duration of gestation	12-13 months	335 days	275-290 days	315 days	150 days
Interval between parturitions	2 years	1-1.5 year	1 year	1.5 years	8-12 months
Days open	8-12 months	9 days	60 days	60-120 days	60-90 days
Number of services/conception	2-4	1-3	1.5-2	2-3	1.2-2
Conception rate	50%	60-70%	75-80%	70%	70-80%
Birth weight	36 kg	45 – 55 kg	28-35 kg	40 kg	2.5-3 kg

Fig. 12: Management systems in farm animals (*Assiut-Egypt, Qassim-KSA, 1988-2013*).

Chapter 4

Clinical Examination of the Female Reproductive Organs

Clinical examination is a fundamental part of the process of veterinary diagnosis. Without a proficient clinical examination and an accurate diagnosis it is unlikely that the treatment, control, prognosis and welfare of animals will be optimized. Rectal examination, if properly conducted, represents the only practical method for examination of the genital organs of large farm animals. It is essential that any veterinarian engaged in conducting dairy or beef cattle reproductive herd health programs possess the skills to accurately and quickly evaluate internal genital organs by rectal palpation. This includes characterization of ovarian structures, recognition of pregnancy and diagnosis of genital organs pathology. The required skills can only be obtained by educating oneself regarding normal and abnormal findings, by extensive practice and experience in rectal palpation and by constant, careful evaluation of palpation records to assess accuracy. Bovine rectal palpation skills are required to diagnose pregnancy, stage the estrous cycle, diagnose internal genital organs pathology, assess previous treatment of internal genital organs pathology, to transfer fertilized ova between donors and recipients, and to artificially inseminate cows.

Rectal palpation of the uterus and its contents is the method of choice for pregnancy diagnosis and for estimation of the stage of pregnancy. The most important factors involved in uterine palpation are proper anatomical orientation and a thorough methodical evaluation of the entire length of the uterine horn. Before the actual examination is performed, the breeding history of the cow should be reviewed, including the date of the last calving, number of services, and information on any pathologic or disease condition previously affecting the reproductive organs.

Gray-scale B-mode ultrasonography is the most profound technological advance in the field of large animal research and clinical reproduction since the introduction of trans-rectal palpation and radioimmunoassay of circulating hormones. It is hard to imagine that many discoveries and procedures related to ovarian, uterine and fetal function that we use today would have been considered without the development of real-time ultrasound. The area that has arguably benefited more from the development of ultrasound technology than any other area is reproduction in large animals. In many cases, rectal palpation has been replaced by transrectal ultrasonography for pregnancy determination, and diagnosis associated with uterine and ovarian infections. In addition, ultrasonography has added benefits such as fetal sexing, early embryonic detection and is less invasive than rectal palpation. From a research standpoint, ultrasound has given us the ability to visually characterize the uterus, fetus, ovary, corpus luteum, and follicles. More accurate measurements of the reproductive organs has opened doors to new areas of research and validated or refuted data from past reports.

Characteristicsfeatures, methods of restrain, and technique of trans-rectal examination in large farm animals are shown in Table (4) and Figs. (13-15).

4.1. Clinical Examination of the Reproductive Organs in Female Camels

The reproductive organs of the female camels can be easily examined manually or using ultrasonography. First of all, the camel needs to be restrained either in stocks or in the sitting position either on the ground or on special tractors (Figs. 13A,B). The rectum is then emptied of feces before the transducer is introduced and rotated in the rectum over the top of the ovaries and uterus. After proper restraint and wearing of proper clothing and also proper lubrication, the operator must make a cone of his hand and push it inside the rectum. The anal sphincter dilates and the hand enters inside the rectum. The feces must be removed without taking out the hand completely. The genital organs lie usually on the pelvic floor in non-pregnant or during early pregnancy beneath the rectum and in the abdominal cavity during late gestation. The genital organs can thus be palpated indirectly by placing the hand in the rectum. The palpator must stop moving his arm during a peristaltic wave (while still keeping his hand inside the rectum) wait for 1-2 minutes and then start palpation again when the peristalsis has subsided. The ballooning of rectum can be easily removed, the operator must catch a pinch of rectal mucosa and move his hand back and forth (known as back racking) without completely taking it out. This will push the air inside, to the exterior and the rectal mucosa will then be closely over the operator's hand. The genital organs can be located by sliding the hand in an arc like fashion from dorsal to ventral side. The genital organs can be pulled caudally when located at the pelvic brim or further, by retracting the broad ligament or hooking the inter-cornual ligament by the index finger (Fig. 14A). When the pregnancy is beyond 90 days this cannot usually be done and the operator has to move his hand further in the rectum, so as to locate the intra-abdominally placed uterus and palpate other diagnostic features of pregnancy. The corpus luteum formed on the camel ovary (ovulation is induced by mating) persists and is necessary for the entire gestation. The persistence of the corpus luteum is one of the earliest sign of pregnancy as otherwise; the luteal phase is very short. The corpus luteum is out of reach by day 90 of pregnancy. The left uterine horn is inherently longer than the right horn and this must be kept in mind when making pregnancy diagnosis in female camels. The earliest detection of uterine change (increase in diameter and appearance of fluctuation) is palpable at about 40-15 days. Between 60-70 days, the left uterine horn is increased about twice to its non gravid size, has a thin wall and fluctuates.

4.2. Clinical Examination of the Reproductive Organs in Mares

4.2.1. Restrain of the mare

The level of restraint needed depends on experience of the handler, and temperament of mare, and quality and quantity of help available. Examination is most difficult in young undisciplined mares handled by amateurs, and easiest in old brood mares handled by experienced personnel. The presence

of a foal at foot may make examination of mares more difficult. No method of restraint is ideal. Some mares are vicious and kick when handled behind; these are uncommon. Most mares are apprehensive when examined for the first time and may need more restraint than subsequently. Most mares tend to walk forward or move sideways if only loosely restrained during examination. The presence (or absence) of the mare's foal, or separation from a companion may make the mare uneasy, as can the sight of unfamiliar objects (clinician's protective clothing, coloured sleeves, lubricant bottles, etc.). Examining mares in a field is made more difficult and more dangerous by the presence of other inquisitive horses.

Stocks are probably the best method of restraint but: the mare may not enter easily, especially for first time; the mare may be uneasy about being confined, especially initially; mares may try to jump out, especially to join a companion or foal (it may be possible to put the foal in the stocks with the mare, otherwise the foal is best placed directly in front of the stocks); ensure that the back panel of the stocks is low; stocks should be readily dismantleable as occasionally mares become cast (Fig. 13D,E,F).

A twitch is a very useful method of restraint and may be the only form necessary for most mares, but: some owners resent the application of a twitch to their mare; some mares are very difficult to twitch; some mares won't move when twitched, so position them correctly before hand; some mares try to go down when twitched tightly; twitching will not always control a willful mare; humane twitch is easy to apply and leaves no mark on the nose. Under field condition, using hobbles methods is a more practical one (Fig. 13D).

4.2.2. Approach of the clinician

Avoid sudden movements and loud noises, but try to converse with helper in an even voice, or hum or whistle. *When mare is not in stocks*, *approach her from the side*, put one hand on the back, run it to base of the tail, grasp tail and pull it to one side. At this stage, mare's temperament and effectiveness of restraint will become apparent. Feel for discharge (wet or dried) on tail, and inspect perineum. For manual examination *per rectum* or *per vaginam*, the arm can be inserted initially without the operator standing directly behind the mare. As examination proceeds, more of the clinician's body is behind the mare, but at this stage her likely reaction has been anticipated. For speculum vaginal examination, it is most convenient for an assistant to hold the tail (in a gloved hand) to allow the clinician a free hand to take the vulval lips apart.

4.2.3. External examination

This may reveal: normal vulva – nearly vertical in position, no distortions (scarring) or discharges; sunken anus (due to old age, poor condition) – common in Thoroughbreds and causes cranio-dorsal displacement of dorsal commissure of the vulva. This encourages contamination of the vulva and vestibule by faeces, and predisposes to pneumovagina; mare with flat-crouped conformation – these mares often have a sunken anus and a resultant angulation of the vulva; vulva already sutured (Caslick's operation) to prevent pneumovagina; lateral or dorsal tears to vulva; small vesicles or ulcerated areas due to coital exanthema (herpesvirus 3). Vulval discharge should not be confused with

small depigmented areas, which are common. It varies from sticky moistness at ventral commissure to frank discharge (wet or dried) on thighs and tail. A small amount of moisture is normal during oestrus, especially after covering, when a temporary purulent discharge may be seen; yellow urine stain (usually dry) on ventral commissure of vulva. Wet vulva usually denotes oestrus (but not always present) – due to increased urinary frequency when showing (winking).

4.2.4. Manual examination *per rectum*

Due to the lateral position of the ovaries (Fig. 14B), one-handed rectal examination makes accurate palpation of the right ovary for the right-handed examiner (and vice versa) difficult if the mare is not well restrained. Wear a glove and use adequate lubricant. Mare usually resents passage of hand most and then the elbow. Completely evacuate rectum of faeces, and feel for uterine horns lying transversely in front of the pubis. Follow these laterally to the ovaries which are cranial to the shaft of the ileum. Always try to have the hand cranial to the structure which is to be palpated to allow sufficient rectum for manipulation. Do not stretch rectum laterally if tense; do not resist strong peristaltic contractions – otherwise rectum may tear (especially dorsally, i.e. not adjacent to examiner's hand). If the rectum is ballooned with air, feel forward for peristaltic constriction and gently stroke with a finger to stimulate contraction. Ovaries often lie lateral to broad ligament and are difficult to palpate. They must be manipulated onto the cranio-medial aspect of the ligament for accurate palpation. Uterus is very difficult to palpate in anoestrus, easier during cycles and easiest during early (up to 60 days) pregnancy, due to increasing thickness and tone of the uterine wall. Cervix is palpated by sweeping fingertips ventrally from side to side in mid-pelvic area. It is easiest to feel during the luteal phase, but more difficult during oestrus and anoestrus.

4.2.5. Visual examination *per vaginam*

Clean perineum and vulva with toilet paper, clean water or weak disinfectant. Moisten or lubricate speculum. After introducing speculum through vulval lips, push cranio-dorsally to clear brim of pubis. At this point there is often considerable resistance at the vestibulo-vaginal junction (occasionally the speculum tries to enter the urethra). When fully inserted (30 cm), you could view the vaginal walls and cervix. Make evaluation quickly, because artifactual reddening can occur following contact of speculum or air with vaginal wall. Evaluate shape, size, position, patency and colour of cervix and vaginal wall.

4.2.6. Manual examination *per vaginam*

Clean vulva and insert lubricated gloved hand. Vagina should be dry in luteal phase and anestrus, moist in estrus, sticky mucus in pregnancy. Palpate cervix for shape, size and patency of canal. You may detect adhesions or fibrosis in the cervix. Do not force finger along cervical canal if there is a possibility of pregnancy. Mare's cervix will allow gentle dilation, without causing damage, at all stages of the reproductive cycle. Manual examination may not be possible if mare's vulva is sutured excessively tight.

4.2.7. Ultrasound examination *per rectum*

Safety is paramount and mares are ideally restrained in suitable stocks. Foals are best positioned either in front of the stocks or within the stocks adjacent to the mare. The rectum should be emptied of faecal material to ensure a good contact between the transducer and the rectal wall. Attempts to manipulate the transducer when the rectum is filled with faecal material may result in tearing of the rectal wall. Should the mare strain during the examination the transducer should be withdrawn.

Imaging technique

The examination should be performed out of direct sunlight, since this can hinder interpretation of images on the ultrasound screen. The ultrasound transducer is usually held within the rectum in the sagittal (longitudinal) plane during imaging. The vestibule and vagina lie within the pelvis in the midline; these structures can be imaged with ultrasound but are indistinct. The cervix is located cranial to the vagina approximately 20 cm cranial to the anal sphincter and can be identified as a heterogeneous, generally hyperechogenic, region with a rectangular outline. The uterus is roughly T- or Y-shaped; therefore when using a linear ultrasound transducer the outline of the uterine body generally appears rectangular (the transducer is in a sagittal plane) whilst the outline of the uterine horns appears circular (the transducer whilst orientated in the sagittal plane is positioned in a transverse plane with respect to the uterine horn). The uterus has a central, homogeneous, relatively hypoechoic, region surrounded by a peripheral hyperechoic layer. The echogenicity of the endometrium and the uterine cross-sectional diameter vary during the oestrous cycle; during oestrus the diameter increases and the uterus becomes increasingly hypoechoic, with central radiating hyperechoic lines which are typical of endometrial oedema. The proximal uterine horns are of smaller diameter than the uterine body. The ovaries can be located by tracing the uterine horns laterally. Various sections of the ovaries are usually examined by rotation of the transducer; sections are usually taken from a medial position, and sequential sections of the ventral, mid, and dorsal portions of the ovaries are examined. Ovaries usually contain follicles (which are anechoic), and may contain luteal structures (which are relatively echogenic – varying shades of greywhite); the ovarian stroma may be difficult to appreciate since it may be surrounded by these structures, although it is generally hypoechoic in appearance. *Endoscopy*: the cervix is easily breached during oestrus in most normal mares. Inflation of gas into the uterus aids navigation of the endoscope and identification of structures. The body of the uterus, bifurcation, uterine horns, which narrow towards the tips, and the oviductal papillae are easily identified.

4.2.8. Pre-breeding evaluation of the mare

Examiner should:

1. Obtain the mare's previous breeding history
2. Assess her physical condition, general health and perineal conformation
3. Culture swab samples collected from the vestibule, clitoral fossa and sinuses

4. Examine the posterior organs per vaginam using a speculum, and collection of endometrial swabs for bacterial culture and stained smear
5. Examine the vagina manually
6. Examine the reproductive organs by rectal palpation
7. Examine the reproductive organs by trans-rectal ultrasonography
8. Obtain endometrial biopsy
9. Examine the endometrium endoscopically
10. Obtain peripheral venous blood sample for hormone/chromosome analysis

4.2.9. Clinical examination of the vulva

In the normal mare the vulva provides an effective barrier to protect the uterus from ascending infection. If the vulval seal is incompetent, pneumovagina may occur and the reproductive organs can become infected. The initial vaginitis may lead to cervicitis and acute endometritis resulting in subfertility. Caslick was the first to point out the importance of this condition in relation to genital infection. Defective vulval conformation can be (1) congenital, which is very rare or (2) acquired, which is due to (a) vulval stretching following repeated foalings, (b) injury to perineal tissue or (c) poor body condition. Older, pluriparous mares are more commonly affected with pneumovagina. However, young mares that are in work and have little body fat and/or poor vulval conformation can develop pneumovagina. In some mares, pneumovagina may only occur during estrus when the perineal tissues are more relaxed. A `Caslick index' has been described in an attempt to determine which mares require treatment, but its use is not widespread. Some mares make an obvious noise whilst walking, but in other mares the diagnosis may be more difficult. The presence of frothy exudates in the anterior vagina on examination with a speculum is pathognomonic. Rectal palpation of a ballooned vagina or uterus from which air can be expelled confirms the diagnosis. Real-time ultrasound examination of the uterus may reveal the presence of air as hyperechoic (white) foci. Cytological and histological examination of the endometrium may demonstrate significant numbers of neutrophils and eosinophils indicative of an endometritis. Treatment should be directed at correcting the physical pneumovaginitis and concurrently treating the acute endometritis. The former can be done surgically by Caslick's operation. However, when the angle of the vulval surface relative to the vertical is the primary deformity, Caslick's operation is ineffective, and perineal resection should be used to achieve a satisfactory vulval conformation.

4.2.10. Clitoral Swabbing

Before the breeding season, swabs may be taken from the clitoral fossa and clitoral sinuses (only the central sinus may be obvious), and the vestibule. The perineal area of the mare should not be cleaned except for the removal of gross contamination of the vulva with faeces using a dry paper towel. A protective disposable glove should be worn by the veterinary surgeon on the hand used to evert the ventral commissure of the vulva and expose the clitoris. The swabs should be placed in

transport medium, clearly labeled with the mare's name and sent to a reputable laboratory. It is important to penetrate the clitoral sinus, and therefore a large swab tip should not be used. Swabs are cultured aerobically on blood and MacConkey agar particularly to screen for the presence of K. pneumoniae and P. aeruginosa. Microaerophilic culture on chocolate blood agar (with and without streptomycin) may also be done for the detection of CEMO.

4.2.11. Clinical examination of the vagina

Vesicovaginal reflux, also known as urovagina and urine pooling, is the retention of incompletely voided urine in the vaginal fornix due to an exaggerated downward cranial slope of the vagina. Pneumovagina from a defective vulval conformation also predisposes to the condition. Transient urine pooling, which is sometimes found in postpartum mares, usually resolves after uterine involution has occurred. Uterine infection with an accumulation of exudates in the vaginal fornix can be confused with the condition. It can be treated surgically by vaginoplasty (perhaps more correctly termed caudal relocation of the transverse fold, as surgical intervention is in the vestibule), urethral extension or perineal resection. Vaginal bleeding from varicose veins in the remnants of the hymen at the dorsal vestibulovaginal junction is occasionally seen in older mares, particularly during oestrus. Treatment is not usually necessary as the varicose veins normally shrink spontaneously, although diathermy can be used. Manual vaginal examination of maiden mares often reveals the presence of hymen tissue which generally breaks down with pressure. A complete persistent hymen can also occur which can result in the accumulation of fluid within the vagina and uterus due to impaired natural drainage. Sometimes the hymen may be so tough that it can only be ruptured using a guarded scalpel blade or scissors. The small incision can then be enlarged using the fingers and hand. Rarely, failure of proper fusion of the Mullerian ducts may result in the presence of dorsoventral bands of fibrous tissue in the anterior vagina and fornix. They do not interfere with fertility and are easily broken down manually.

4.2.12. Clinical examination of the cervix

The cervix, whilst forming an important protective physical barrier to protect the uterus, must also relax during oestrus to allow intrauterine ejaculation of semen at coitus and drainage of uterine fluid. A cervicitis is usually associated with endometritis and/or vaginitis. Fibrosis of the cervix often occurs in older mares, particularly maiden mares. Adhesions of the cervix arise from trauma at parturition or mating; they can be broken down manually, but this must be done daily to prevent recurrence. Artificial insemination has been used successfully in mares with an abnormally narrow cervix. Impaired cervical drainage of uterine fluid can predispose to chronic endometritis. Cervical lacerations may need surgical repair if severe. Developmental abnormalities of the cervix have been described; these include aplasia and a double cervix.

4.2.13. Endometrial swabs and smears for diagnosis of endometritis

A diagnosis of endometritis can be made by collection of concurrent endometrial swab and smear samples during early estrus for bacteriological culture and cytological examination, respectively. This allows time for resolution prior to mating, and maximizes the chances of pregnancy.

The ideal technique should ensure that the swab enters the uterus and collects bacteria from only the uterine lumen.

Two methods can be used:

1. A non-guarded endometrial swab on a sterile extension rod is carefully passed via a sterile speculum through the cervix into the uterine body and, after withdrawal, is placed in transport medium. A second swab is taken immediately afterwards for the endometrial smear.

2. A guarded swab is passed into the uterine lumen using a sterile speculum or enclosed in a disposable plastic arm-length glove. The swab tip is exposed only when it is in the uterine lumen. A second swab for cytological examination should again be taken. Swabs for culture should be plated on blood and MacConkey agar, and incubated at 37^{o}C for 48 hours. Cultures should be examined at 24 and 48 hours. An air-dried smear is made by gently rolling the second swab either on a Testsimplet (Boehringer Corporation), which is a pre-stained slide or a clean dry microscope slide. The smear can be differentially stained with a rapid stain such as Diff-Kwik (American Hospital Supplies). The stained smear should then be examined for the presence of inflammatory and endometrial cells the latter confirming contact of the swab with the endometrium Interpretation: A positive culture result, with no evidence of inflammatory cells in the smear (usually neutrophils), is likely to be due to contamination during collection. Diagnosis is based on the presence or absence of significant numbers of neutrophils in the smear. Very rarely, neutrophils can be detected, usually at the `foal heat' or the first oestrus of the breeding season in maiden mares, although there is no endometritis (Fig. 15).

4.2.14. Endometrial biopsy

In some cases, endometrial biopsy may be a useful diagnostic aid. The technique involves the insertion of a biopsy instrument through the cervix and into the uterus. With the biopsy instrument in the uterine lumen, a gloved hand is inserted into the rectum to allow manipulation of the instrument into the desired position. The sample is taken by closing the jaws of the instrument and tugging sharply. To avoid damage, the tissue is carefully transferred into a fixative solution by dislodging it from the jaws of the punch with a fine hypodermic needle. The instrument most commonly used today is the Yeoman (basket-jawed) biopsy forceps, ideally 60cm to 70cm in length, with which tissue specimens 2 x 1cm (about 0.2% of the whole endometrial surface) are obtained. If the uterus appears normal on palpation, the sample should be taken from one of the areas of embryo fixation, i.e. the uterine horn--body junction on either side. Single samples are usually representative of the entire endometrium. If the uterus is abnormal on palpation per rectum, biopsy samples should be taken from both the affected area and a normal area. Biopsy specimens should be fixed in Bouin's fluid followed by sectioning and staining with haematoxylin and eosin. The endometrial biopsy sample should be sent to a laboratory that is experienced in evaluating samples.

4.2.15. Detection of intraluminal uterine fluid using transrectal ultrasound imaging

Transrectal ultrasonography provides a non-invasive method of assessment of the uterus. If no free fluid is detected during estrus, then acute endometritis as detected in cytology is absent in 99% of

cases. Free fluid does not indicate inflammation. Endometrial cytology and culture fails to detect sterile fluid accumulations.

4.3. Clinical examination of the reproductive organs in cows

For most dairy cows, the restraint required to perform a rectal palpation is minimal. Usually a head gate or halter is sufficient (Fig. 13G). However, certain cows may require additional restraint such as a nose lead or having their tail forced firmly dorsally and cranially "jacking the tail" to decrease sudden side to side movements or kicking, both of which may cause injury to the examiner. Stocks are optimum for examination (Fig. 13H). The examiner should wear a disposable plastic sleeve or latex obstetrical sleeve, and the sleeved hand and arm should be well coated with a non-irritating, water soluble lubricant. Disposable plastic sleeves may be worn inside out to keep the sleeve seam out of contact with the rectal mucosa. Some practitioners tear off the fingers of the disposable plastic sleeve and wear a latex exam glove over the hand to increase tactile sensitivity and decrease irritation to the cow's rectum. All jewelry must be removed from the hand and arm to be inserted into the cow's rectum and the examiner's finger nails should be trimmed and clean.

Pelvic Cavity

Because palpation of the uterus per rectum relies solely on the sense of touch, it is important for the examiner to be familiar with the bony landmarks within the pelvic cavity. The importance of these landmarks will become clear as they are referred to in the pages to follow Placement of the gloved and lubricated hand into the rectum is achieved by holding one's hand in the shape of a cone and inserting the hand through the anus and into the rectum using a slow rotating motion with firm, but gentle pressure. Advancing the hand in this manner often stimulates defecation. However, additional fecal material may need to be removed from the rectum manually. This may be done by cupping the hand and gently raking feces caudally toward the anus, allowing them to pass out of the anus beside the arm without removing one's hand. Occasionally during an exam, the rectum may become filled with air, causing the walls of the rectum to become distended. When this occurs, palpation of internal structures becomes virtually impossible. Normal peristalsis will expel this air, but the process may be expedited by reaching forward and hooking one's fingers through the peristaltic constriction band and gently pulling it caudally toward the anus, allowing air to escape beside the arm. It is common for the examiner to encounter peristaltic waves within the rectum during an examination period. These are felt as a constriction of the rectum that advances toward the anus. The hand should never be forced through one of these waves. Instead, they should be allowed to pass over the hand and arm before proceeding with the examination. In small heifers, this too may be contraindicated, as even this amount of strain on rectal tissues may result in laceration. If fresh blood, or clotted blood in excess of 15 ml, is observed coming from the rectum during an exam, it is highly indicative of rectal rupture and requires immediate cessation of the exam and supportive therapy of the cow. Such incidences may result in sepsis, or in adhesions that can compromise reproductive function. This poses a serious health risk to the cow, and may result in a decision to cull. In general, vigorous examination should be avoided since it not only increases the risk of harm to the cow, but also induces more peristaltic waves and rectal tone, making palpation more difficult. The examiner will find that gentleness; care and patience will reduce injury to the cow, and increase the efficiency of the procedure.

Cervix

Once the hand and arm are in the rectum, and feces and air have been removed, the process of uterine retraction and examination may proceed. The first step is to locate the cervix. Usually the cervix lies on the midline of the floor of the pelvic cavity, but may be displaced laterally by a full bladder or a short broad ligament. To find the cervix, slide the hand down one wall of the pelvic cavity, and across the pelvic floor of the opposite wall, feeling for a firm, cylindrical, somewhat irregular structure lying parallel with the axis of the cow. This structure is the cervix, and should not be confused with any other structure in the pelvic cavity. Once located, the cervix can be grasped and, in the pregnant cow (or cow in early pregnancy), it should be freely movable. At this time the examiner should note the size, shape, form, consistency and position of the cervix. The annular folds of the cervical mucosa can be appreciated. Depending on the age and parity of the individual, the cervix of a normal cow can range from 5 to 12 cm in length and 2 to 6 cm in diameter, and changes little over the course of the estrous cycle. In certain breeds (e.g. Guernsey, Shorthorn), the cervix itself may extend over the pelvic brim and lie partly in the abdominal cavity. In the pregnant cow, the cervix becomes more enlarged.

Uterine retraction is initiated by grasping the cervix and pulling it dorsally and caudally. One attempt should bring the uterine horns and broad ligament above the pelvic brim, but in some larger breeds or individuals with larger organs, this may need to be repeated. The uterus may then be held in place by putting the thumb under the uterine body and suspending it against the pelvic wall, leaving the fingers free to locate the broad ligament. Alternative, the hand is cupped and hold the uterine horns backwards (Fig. 14C).

While holding the uterus in place with the thumb, hook the remaining four digits around the anterior edge of the broad ligament, at the angle between the ovarian tip of the uterine horn and the ovary. The broad ligament, at this point, should be slightly taught as the result of the cervical retraction performed previously. However, it should be noted that this part of the procedure often proves to be one of the most challenging and problematic steps for the beginner.

With the fingers in contact with the broad ligament, run them ventrally and medially along its anterior edge to locate the uterine horn to which the ligament is attached. With short, gentle movements of the fingers, gather the horn into the hand working medially toward the uterine bifurcation. This is another step which is often difficult for the beginner who tends to lose hold of the horn.

Bifurcation

Once the fingers have reached the base of the uterine horn, the bifurcation of the horns can be appreciated. At this time, the two intercornual ligaments can be palpated: The dorsal intercornual ligament which is the smaller and thinner of the two, and the ventral intercornual ligament which is larger and thicker. The ventral intercornual ligament will be used for retraction of the uterus, the dorsal one being too fragile for the task. Hook the tip of the middle finger under the ventral intercornual ligament, and pull the uterus dorsally and caudally into the pelvic cavity, reflecting the

uterus back on itself. In order for the uterus to remain in place and allow effective examination of the uterine horns, the ventral intercornual ligament should be at the level of the ischiatic arch, the cervix should be upside down, and the uterine horns should be entirely within the pelvic cavity. This procedure works well in nonpregnant cows and in cows pregnant for less than 50 - 60 days. However, in cows pregnant for more than 65 days, or who have pyometra, hydrometra, or other uterine abnormalities causing the uterus to be enlarged, heavy and possibly friable, this procedure may not be effective. Instead, such instances may require that the hand be passed beneath the uterine horns so that they may be lifted into the pelvic cavity. In later pregnancy, or in a grossly enlarged uterus, retraction is not possible. In some animals, especially nulliparous or primiparous ones, the uterus can be retracted simply by reaching forward and by directly locating the ventral intercornual ligament and reflecting the uterus caudally. This method, however, is not routine, and often fails. At any point during the retraction process peristaltic waves can occur and interfere with palpation, causing the examiner to lose hold of the uterus. When such peristalsis occurs, one can keep the uterus in place by cupping one's hand and pressing the uterus to the floor or wall of the pelvic cavity. Again, gentle manipulations will stimulate less peristaltic activity than vigorous palpation. As one becomes more adept, the process can be achieved in less time, which also will decrease peristaltic interference. It has been noted that wintering cows that are on dry, preserved feed have less peristaltic activity and rectal tone than cows on lush pasture in warmer seasons.

Once complete retraction of the uterine horns has been achieved, a thorough examination should be performed. Starting at the base of the horn at the bifurcation and working toward the tip, examine each horn for size, form, consistency, tone and contents. It is important to be certain that the full extent of both uterine horns have been examined. Palpable qualities of the uterine horn change with reproductive and disease status of the cow. During a normal estrous cycle, uterine tone will begin to increase a few days before the onset of estrus, becoming fully toned at estrus. This condition will persist for about 2 days, and then tone will decrease as the cow enters the luteal phase of her cycle. During the luteal phase, the horns become soft and flaccid. Therefore, it is easier to identify and palpate the uterine horns during or close to estrus. Diagnosis of pregnancy is another important function of rectal palpation. In order to diagnose a pregnancy by palpation, one must detect one or more of the "positive signs of pregnancy": **1**. Fetal membrane slip, which can be appreciated from about 30 days to term; **2**. Amniotic vesicle, which is palpable between 30 - 65 days of pregnancy; **3.** Placentomes, palpable from about 75 days to term, and **4.** Fetus, felt from about 65 - 70 days to term. Some consider fremitus (palpable turbulence) in the uterine artery a positive sign of pregnancy, since it is rarely present in a non-pregnant individual. Fremitus is palpated during pregnancy in the uterine artery ipsilateral to the pregnant horn. It should be noted that prolonged and vigorous palpation of a pregnant cow can increase the risk of early embryonic death. This occurs especially as a result of attempts to palpate the amnionic vesicle and the fetal membrane slip. Fewer problems are observed with palpation of fluctuation in the uterine horn. It is best, when palpating a cow in early pregnancy, to do so gently, and recheck her after 58 -60 days for confirmation. Uterine size decreases quite rapidly after parturition. Normally, the uterus is small enough to allow palpation of its full extent by 7 - 10 days post-partum. It should be at normal size by about 25 days, and should be completely involuted by 42 - 46 days. The cervix returns to normal at an even faster rate.

Ovaries

When thorough examination of the uterine horns is complete and the cow has been determined to be non-pregnant, or is pregnant but there is concern that there has been fetal death, one may proceed with palpation of the ovaries. The ovary is located by finding it in relation to the tip of the uterine horn, or by recapturing the broad ligament and locating the ovary which is suspended by the mesometrium. Once the ovary is found, it is held so that the ventral "free" border faces dorsally, while the dorsal "attached" border rests in the hand, placing the mesovarium between the middle and ring fingers. The surface of the ovary is then explored using the forefinger and the thumb, allowing the examiner to evaluate ovarian size, consistency, presence of functional structures, and any abnormalities. Characteristics of the ovary vary between ovaries in the same individual, and between individuals, ages, and breeds. In the prepuberal heifer, the ovaries are small and smooth. In postpuberal heifers and cows, ovaries that are smaller than 2 cm in length by 1.5 cm in width by 1.5 cm in thickness can be suspected of being hypoplastic or atrophied. Normal ovaries vary in size and shape over the course of the estrous cycle, on average ranging from 2 - 4 cm long by 2 - 3 cm wide by 2 - 3 cm thick. Some increase in ovarian size is associated with developing follicles, but the greatest changes in sizes are due to the presence of the corpus luteum. If an ovary is found to be abnormally large, this may be due to a follicular or luteal cyst or to neoplastic change.

4.4. Clinical Examination of the Reproductive Organs in Buffalo-Bows

It is better to restrain the buffaloes in chutes (Fig. 14I). It is not recommended to catch the animal from the nasal septum or to hold the animal from the inguinal fold, she becomes more vigorous. Scratching the back or knocking on the horns with solid objects may help to keep the animal quit during examination. Clinical examination of buffalo-cows resembles to large extend that of the cattle, but buffaloes are more vigorous, the genital organs are mostly located in the pelvic cavity in the non-pregnant status, and the ovaries and their structures are smaller. Manipulation and examination of the reproductive organs of buffaloes via rectal palpation is possible because of several anatomical features:

1) As in cattle, the reproductive organs of the bovine are suspended from the dorsal body wall by the broad ligament. The posterior part of the organs (vagina, cervix and, sometimes part of the uterus) is located in the pelvic cavity (formed by the two pelvic bones) and the anterior part of the organs hangs over the pelvic brim (uterus, uterine tube, ovaries and, sometimes part of the cervix) and is suspended in the abdominal cavity. Moreover, the organs are located immediately below the rectum.

2) The rectum of the buffalo-cow is large enough to allow insertion of the hand and arm of the palpator. Moreover, the rectal wall is sufficiently pliable to allow one to grasp and identify reproductive organs structures through the rectal wall. 3) The rectal wall of the buffalo-cow is strong so that tearing, while possible, is rare. Note the situation is different in other species, notably the mare, where the rectal wall is more subject to damage. Nonetheless, care should be taken when performing rectal palpation of cattle because the rectum can be torn or otherwise damaged.

Ultrasonography can be effectively employed to record the exact size and echotexture of the buffalo genitalia during different stages of estrous cycle.

4.5. Clinical Examination of the Reproductive Organs in Ewes and Does

Breeding History

A complete history should be an important part of the breeding soundness examination (BSE) because of the inaccessibility to the majority of the reproductive organs to palpation or visual inspection.16,17 Information regarding the intention of the owner in utilization of these goats for production (meat, milk, and fiber), brush control, or as a companion animal should be gathered. These include housing (pasture, dry lot, backyard, or barn); Nutrition (pasture, concentrate, hay, silage, trace minerals); breeding Management (natural or artificial insemination), time of the year (season), hormonal manipulation, male-to-female ratio, number of males in a pen or pasture, fertility of the male used, male libido, and prior history of urethral obstruction. In case of artificial insemination, information obtained should include source of the semen, evaluation of the frozen semen, heat detection, experience and success of the inseminator, bred once or twice during estrus, evaluation of the vagina and cervix on speculum examination at the time of AI, presence or absence of mucus at the time of AI. Other information include length of estrous cycle, interestrous interval, duration of estrus, reaction of the female to the male, number of teasers checking estrous behavior, frequency of heat detection.

Physical Examination

This includes **c**urrent body condition, conformation, lameness, polled or horned, and eyes

External and internal genitalia examination

Visual examination of the perineal area should include evaluation of the anogenital distance.

Vulva

Examine the lips of the vulva, then part the lips of the vulva and evaluate the clitoris. Any lesions on the vulva (scabs, ulcers, pustules, etc.) should be noted.

Vagina

Speculum examination is made of the walls of the vagina, vestibule, and cervix. Rule out any urine pooling in the vagina and also discharge. If discharge is present, determine the source (vagina, cervix, or through the external os of the cervix).

Cervix

Examine the external os and area around the opening (fornix). Digital examination along with speculum/endoscopic evaluation could be done to evaluate the vagina, vestibule, and cervix and to rule out persistent hymen, lacerations, and adhesions. During the period of estrus in a doe, there is clear mucus discharge seen in the anterior vagina, later turning to cloudy (milky) toward the end. This is normal discharge and does not require treatment.

Reproductive Ultrasonography

Ultrasonography has been used for evaluating the reproductive organs in small ruminants. Goats are scanned transabdominally either at the right or left inguinal region or may be scanned transrectally using a probe adapter to a 7.5-MHz linear transducer. Goats are usually scanned standing, and they tolerate it better than being tipped over in a dorsal recumbency posture. Transabdominal scanning is done on the right side, slightly cranial to the mammary gland, and about 3 to 4 inches from the midline (paramedian), because the reproductive organs are pushed toward the right side by the rumen. For transabdominal scanning, 3- to 5-MHz curvilinear or sector scanning transducers are superior to linear-array transducers; 5- to 7.5-MHz linear transducers have been used transrectally. Earlier pregnancies around 20 to 25 days may be seen best with a transrectal approach, preferably in a dorsal recumbency position.

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APPENDIX: Tables and Figures

Table 4: Characteristics features of trans-rectal palpation in large farm animals and methods of restrain

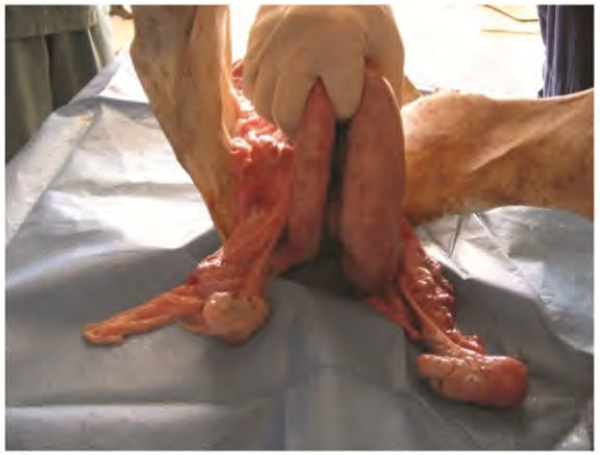
	Female camel	mare	cow	Buffalo-cow
Animal restrain	sitting - stanchion	hobble - stanchion	stanchion	stanchion
Land-mark	uterus	uterus or ovaries	cervix	cervix
cervix	less distinct	less distinct	well distinct	well distinct
uterus	Pelvic/abdominal	Pelvic/abdominal	Pelvic/abdominal	Pelvic/abdominal
ovaries	pelvic prim	sub-lumber	Pelvic cavity – pelvic prim	pelvic cavity

Restrain of female camels for trans-rectal examination on a transporting truck (A), in stanchion (B), or in sitting position (C).

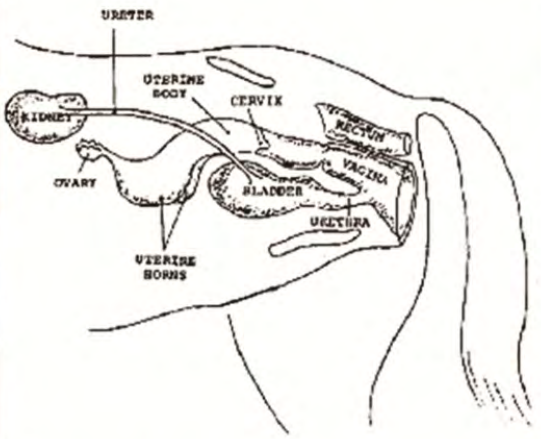
Restrain of mares by hobble method (D), or in stanchion (E, F).

Restrain of cattle by grasping of nasal septum (G) or by keeping in stanchion (H), examination of buffalo in stanchion (I).

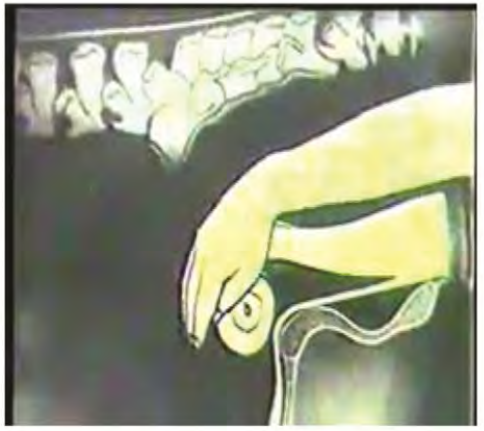
Fig. 13: Methods of animal restrain during rectal examination (*Assiut-Egypt, Berlin-Germany, Ohio-USA, Qassim-KSA, 1988-2013*).



A) Female camel: retraction of the uterus into the pelvic cavity by grasping the bifurcation, then palpate the right horn and the right ovary, then return again to the bifurcation to palpate the left horn and the left ovary



B) Mare: the hand palpates the uterus at the pelvic brim, then runs upward through the horns to the ovaries in the sublumber region



C) Cow, buffalo-cow: making the hand as a cup shape to palpate the uterus at the pelvic brim, then run to the right and left sides to examine the uterine horns and ovaries

Fig. 14: Technique of trans-rectal palpation in large farm animals (*Berlin-Germany, Qassim-KSA, 1997-2011; www.uaex.edu*).

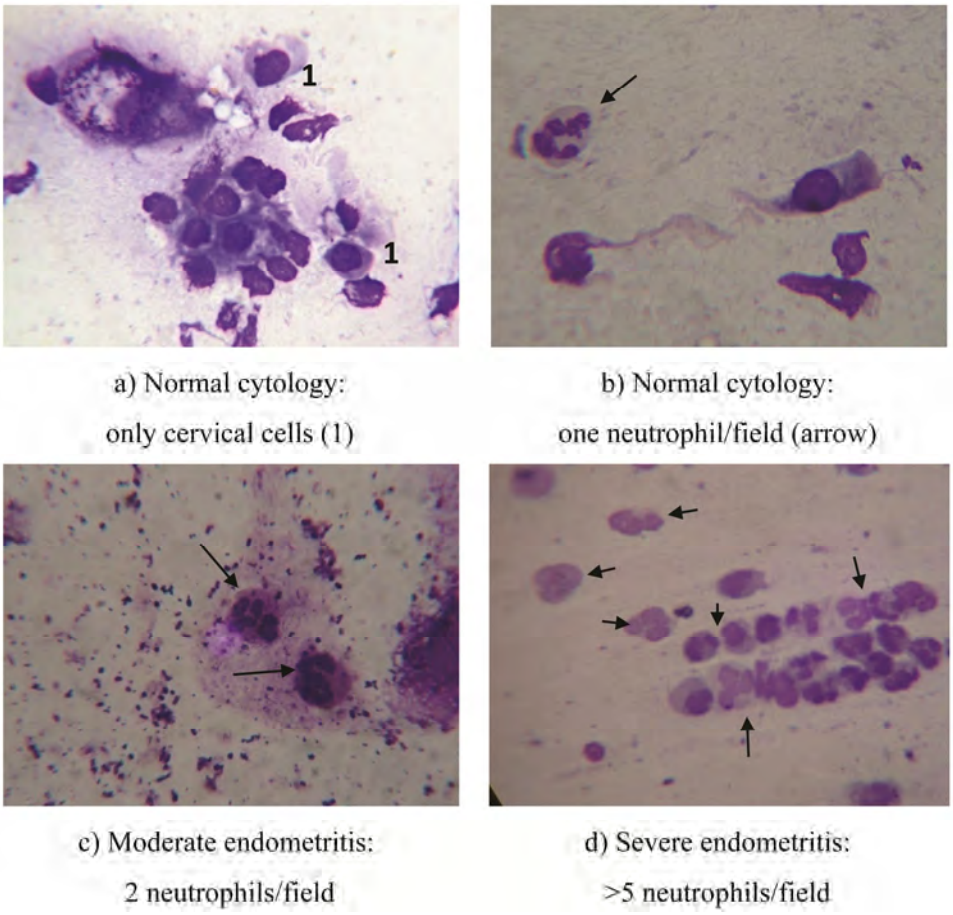


Fig. 15: Cervical cytology in mares, Giemsa stain, X100 (Qassim-KSA, 2012).

Chapter 5

Infertility of the Female Animals

Infertility is a temporary disturbance of reproductive function wherein the animal cannot become pregnant and produce a newborn. Normally a good and healthy animal should give a newborn for every 24, 12, 12, 14 and 8 months interval for female camels, mares, cows, buffalo-cows and ewes and does, respectively (Table 3). If not, it is an economical burden to the farmer since an unproductive animal has to be maintained. Infertility is also due to poor management resulting in delayed puberty and first calving. Sterility is a total loss of fertility and maintaining such animals is a waste and should be culled. Whereas infertility is a temporary loss of fertility can be corrected if diagnosed in the right time and can be assisted. Infertility problems are the common cause for culling in animals due to poor management, feeding and disease protection.

Causes of infertility in different farm animals are shown in Tables (5-9). Bacterial isolates associated with infertile cases in female camels and mares are given in Tables (10-11). The common diseases of the reproductive organs of farm animals are illustrated in Figs. (16-32).

5.1. Infertility in Female Camels

5.1.1 Causes

Over six breeding seasons, a total of 3447 female camels were examined by the author and his co-workers for the prevalence of reproductive disorders in Saudi Arabia. Based on history, animals were categorized as repeat breeder (*n*=2343), refused mating (*n*=573) and early embryonic death (*n*=531). Animals were then exposed for trans-rectal examination, vaginal exploration and trans-rectal ultrasonography. Results showed that endometritis, ovarian hydrobursitis, and vagina and cervical adhesions were the major causes of infertility. Ovarian cysts and ovarian inactivity did not represent major infertility problems. Miscellaneous causes of infertility included anomalies of the genital organs, hydrosalpinx, and ovarian and vaginal tumors.

Another reason for infertility in female camels could be mating at the wrong time of follicular development i.e. when the follicle is either too small, too large or is regressing as there would not be a fertile oocyte in any of these types of follicles.

Another study for the author and his co-workers was planned to investigate the relationship between the characters of vaginal discharges and the prevalence of uterine bacterial isolation in repeat breeding female camels and to determine the sensitivity of these isolates to antimicrobial and antiseptic agents. Forty five repeat-breeding dromedaries were grouped according to the characters of vaginal discharge into those with no discharges (D0, *n*=7), those with gelatinous (D1, *n*=11), those

with mucopurulent (D2, *n*=11) and those with purulent discharges (D3, *n*=16). Uterine swabs were collected for culture, and bacterial isolates were identified. The obtained isolates were tested against a panel of antibiotics and three antiseptic solutions. A total of 78 isolates were obtained from 39 of the investigated animals, the other 6 animals had no bacterial isolates. The prevalence of bacterial isolates did not differ significantly among groups (85.7% in D0, 90.9% in D1, 81.8% in D2, and 87.5% in D3). *Staphylococcus aures*, *Trueperella pyogenes*, and *Streptococcus pyogenes* were the most prevalent bacteria in female camels with D1, D2 and D3, respectively. Gram positive isolates were highly susceptible to Chloramphenicol, Gentamicin, and Cotrimoxazole, while Gram negative isolates were most sensitive to Gentamicin and Cefotaxime. Concerning antiseptics, bacterial isolates were more sensitive to Acriflavine 0.1% than Lotagen 4% (92.9% vs. 53.6%, P=0.004). None of the isolates was susceptible to Povidone-iodine in concentrations of 1%, 0.5%, and 0.1%.

Ovarian hydrobursitis is a peculiar affection of the ovarian bursa characterized by the accumulation of variable amounts of fluid and encapsulation of the ovary. The condition is suspected when difficulty is encountered during retraction of the uterus and ovarian palpation. In some cases, the uterine tube presents a severe torsion easily identified by palpation. The ultrasonographic appearance of the ovarian-bursa and its contents is variable and depends on the size, ovarian activity and the nature of the fluid within the bursa. The etiopathogenesis of this condition is not well understood but could involve bursal adhesions, uterine infection or predisposing genetic factors. The condition could be due to chronic infection involving *Chlamydia*. Reproductive life can be saved in the case of unilateral affections by surgical removal of the affected bursa and ovary.

A garnulosa cell tumor has been reported by the author in a dromedary camel. The 14-year-old female dromedary camel was examined for failure of conception. Ultrasonography revealed a mass of mixed echogenicity involving the left ovary, which was later surgically removed. The cut surface was multilocular. Upon microscopic examination, a mature granulosa cell tumour (GCT) was found. Laboratory examination revealed neutrophilia, increased albumin and blood urea nitrogen and decreased globulin, calcium, phosphorus, and magnesium. Estrogen, progesterone, and testosterone were within the normal values. Teratoma and dysgerminoma has been reported in the dromedary camel but are extremely rare.

5.1.2. Treatment strategies

A total of 480 female dromedary camels affected with endometritis were randomly assigned to receive one of three intrauterine treatments: (i) 100 mL acriflavine 0.1% (group 1, *n*=170), (ii) 100 mL lotagen 4% (group 2, *n*=200), or (iii) 300 mg/100 mL gentamicin sulphate (group 3, *n*=110). All groups received 500 mg cloprostenol IM at infusion. Animals were exposed for breeding 7 d later and received 5000 IU hCG im at mating. The criteria for efficacy of treatment were 90 days non-return rate (90 d NRR) and calving rate (CR). The results showed that the 90 d NRR and CR were significantly influenced by parity, type of uterine infection, regime of treatment, and their interactions. Treatment regimes were approximately equally efficient in treating females with endometritis (90 d NRR were 64%, 53.1% and 53.3% and CR were 58.9%, 49.3%, and 42.5% for groups 1, 2, and 3, respectively). In contrast, regimes differed in treating those with metritis (90 d NRR were 55.6%, 75%, and 28.6% and CR were 31.6%, 54.8%, and 12.5% for groups 1, 2, and 3, respectively). In

conclusion, a regime consisted of intrauterine lotagen infusion and administration of PGF2α at infusion and hCG at mating was more efficient for treating female camels with endometritis.

Twenty eight camels diagnosed with ovarian hydrobursitis were investigated for the effect of unilateral surgical ablation on breeding outcomes. Surgical ablation was carried on 14 cases (treated group), the remaining 14 cases were followed as controls (control group). Both groups were observed for breeding results: 90 days non-return rate (90d NRR) and calving rate (CR). Removed bursae were sent to the laboratory for histopathological investigation. The 90d NRR and CR of the surgically-treated cases were 64.3% and 50%, respectively. None of the untreated cases conceived. These results confirmed that ovarian hydrobursitis causes infertility in dromedary female camels and is associated with inflammatory genital conditions and surgical ablation in unilaterally affected animals presents a potential treatment.

Sixty female camels affected with bilateral ovarian hydrobursitis were divided into treated and control groups (n=30 each). Based on the bursal diameter, females of both groups were subdivided into those having small (< 5 cm), medium (5–7 cm) or large (>7 cm) bursae. Treated group received 20 mg/kg body weight oxytetracycline intramuscular, 4% lotagen intrauterine, and 500 μg cloprostenol intramuscular. Controls did not receive any treatment. All females were observed for 90 days non-return rate (NRR) and calving rate (CR). Antibodies against *C. abortus* were observed in 44/51 (86.3%) of the affected females. The 90 days NRR of the treated and control groups were 13/30 (43.3%) and 0/30 (0.0%), respectively, while the CR were 10/30 (33.3%) and 0/30 (0.0%), respectively. Based on bursal size, the 90 days NRR were 11/15 (73.3%), 2/7 (28.6%) and 0/8 (0.0%) for treated females having small, medium and large bursa, while the CR were 9/15 (60%), 1/7 (14.3%), and 0/8 (0.0%), respectively.

Female camels with vaginal or cervical adhesion might be treated by manual dilatation; however the prognosis is guarded.

5.2. Infertility in Mares

5.2.1. Causes

Most broodmares are relatively normal through the majority of their breeding career, but as they age problems often arise. There are others that start their career as problems which may or may not resolve with time and treatment. The veterinarian in reproductive practice is often presented with these mares which allow him/her to use different diagnostic skills than in some other areas of practice. Sometimes the challenge is in diagnosis, but ultimately the challenge usually lies in managing the mare and her problems to produce a foal. These mares usually present with signs generally referable to one or more of three categories: uterine abnormalities; ovarian and cyclic abnormalities; failure to become pregnant. Uterine abnormalities are the most common cause of reproductive failure in the mare and come in many forms, although most involve inflammation of one form or another.

In a study for the author and his co-workers about the causes of infertility in Arabian mares in Saudi Arabia (*n*=222), endometritis was the common cause of Infertility (44%). Moderate endometritis (2-5 neutrophils/field) was found in 60 mares, while severe endometritis was diagnosed in 39 mares. Among them, intrauterine fluid accumulation was observed in 23 mares and vaginal discharges were observed in 12 cases. *E coli* was the most commonly isolated bacteria (16x). *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Streptococcus equi zooepidemicus*, and *Klebsiella pneumoniae* were isolated from some mares with endometritis. Narrowing or adhesion of the cervix and/or vagina was the second important cause of infertility in Arab mares (16.4%). All affected mares were multiparous with an average age of 14.2±6.1 years (range 5-22 years). It was accompanied with intrauterine pus accumulation (pyometra) in 20 mares. Culture, cytology, and biopsy are major tools useful for uterine evaluation, but the uterus is a dynamic organ that can only be fully evaluated over time with observation through an estrous cycle. Some conditions involving post-breeding endometritis such as delayed uterine clearance and hyper-responders can only be totally evaluated during a breeding cycle. Endometrial cytology is a frequently overlooked method of obtaining quick information on the inflammatory status of a mare. It is all too common for veterinarians to rely on ultrasound examination as the only method of uterine evaluation. Endometritis can result in uterine fluid, but many cases of endometritis never exhibit uterine fluid, and the diagnosis would be missed without culture and/or cytology. Cytology is simple and relatively rapid, easy enough to perform in nearly any clinic laboratory. Intrauterine fluid prior to breeding should be evaluated as soon as possible, especially if the fluid is present in a diestrus mare. Diestrus fluid is almost always a sign of endometritis, usually infection. If fluid is present in the uterus at the time of insemination, the semen will not be damaged if uterine lavage is carried out immediately prior to insemination with a buffered lactated ringers solution or Dulbecco's phosphate buffered saline (PBS), while saline itself is very detrimental to spermatozoa. Following breeding uterine fluid the most frequent causes of uterine fluid are delayed uterine clearance (DUC), hyper-responders, and infectious endometritis. Over time the uterus is repeatedly forced to fight both breeding and environmental contamination. A single breeding introduces hundreds of millions or billions of spermatozoa that are treated as foreign invaders by the immune system, as well as large numbers of bacteria and debris. The normal mare will respond quickly and clean the uterus out within 12–24 hours with an influx of inflammatory cells, removing them and the contaminants through uterine contractions and expulsion or through the lymphatic system. In fact it is normal to see purulent exudates draining from the cervix of a mare 8–12 hours following breeding. This cleaning process requires a normal immune system, an open cervix, normal oxytocin-induced uterine contractions resulting from the release of prostaglandins, and a uterus that is not too far below the brim of the pelvis. Fungal endometritis is often secondary to resolved bacterial infections, although it can be a primary infection, and can be quite challenging to treat. Most fungi grow well on blood agar although yeast may take 48 hours of culture. Filamentous fungi such as Aspergillus can often be successfully treated with uterine lavage with dilute betadine solution as long as vigorous ballottement of the uterus is performed transrectally to dislodge the fungal masses from the endometrium. Yeast infections are much more challenging to treat, as the organisms typically have an intracellular stage that can be quite resistant to treatment. Most treatment regimens need to be continued for at least 7 days and then sometimes repeated after the mare comes into heat again, to result in the elimination of a susceptible organism.

Ovulation abnormalities have become much more recognized since the advent of the use of ultrasound for routine breeding exams. These abnormalities include hemorrhagic anovulatory follicles, unruptured luteinized follicles, and ovarian hematomas. Hemorrhagic and unruptured luteinized follicles appear to be more prevalent in mares suffering from uterine inflammation or infection and mares that are hypothyroid. The classic hemorrhagic follicle appears to grow as a normal follicle until the time of expected ovulation approaches, when the follicle continues to grow, often at an increased rate, and ovulation does not occur. These follicles often reach 6–8 cm in diameter, sometimes larger, and gradually fill with blood as evidenced by their increased echogenicity. Fibrin strands will usually form; crisscrossing the follicle which will gradually decrease in size after the mare goes out of heat. Most of these follicles will have luteal tissue form in the wall and will eventually respond to prostaglandins, although some fibrin, etc. will remain visible in the ultrasound scan of the ovary for some time, giving the appearance of a corpus luteum. Hemorrhagic anovulatory follicles are more common in older mares and late in the season. Mares that have had one cycle with a hemorrhagic follicle are more likely to have another than would a mare that has not experienced such a follicle

Ovarian inactivity was observed in 15.8% cases of the infertile Arab mares. Hard and small ovaries with no significant follicular or luteal structures were observed in these cases. Of them, 18 mares were suckling. The mean body condition score of these mares averaged 1.8. Multiple endometrial cysts of large sizes were observed in 14.1% cases of the infertile mares. The endometrial cysts were found in the uterine body and/or in both uterine horns. All mares were over 10 years old (16.3 years). Ultrasound examination was optimum in observing such cases. Additionally, fiberoptic examination could clearly identify these cases. Pneumovagina was diagnosed in 8.2% cases of the infertile Arab mares. All of these mares were either old, and/or with low body condition score (2.1).

Ovarian tumors were observed in 1.4% of the infertile Arab mares. Based on histopathological examination, adult type granulosa cell tumor (GCT), mixed germ cell tumor, and low grade Sertoli cell tumor were diagnosed. The granulosa cell tumor was transrectally palpated as a large thick walled round structure anterior to the shaft of the right ileum. By ultrasound, it appeared as a large circumscribed structure with a central hypoechogenic area surrounded by an echogenic layer. Many hyperechogenic trabeculae were observed in the central dark area. After surgical removal, the tumor was grossly examined. It appeared as 6.5 cm in diameter, round, smooth, and thick walled structure. The tumor was cystic in cross section; the inner surface was mostly rough and uneven. Hemorrhagic fluid was detected inside the tumor cavity. Microscopically, the ovarian tissues were almost replaced by polygonal neoplastic cells with necrotized eosinophilic stroma. Many cystic spaces were found in the stroma. Mitotic figures with pyknotic nuclei were also detected. The serum estradiol-17β and progesterone levels just before surgical removal were 32.25 pg/mL and 0.03 ng/mL, respectively. On the other hand, the fluid found in the tumor cavity showed high levels of estradiol-17B (501.9 pg/mL) and progesterone (3.19 ng/mL).

The mixed germ cell tumor was palpated transrectally as a very hard structure extended from the left horn to the caudal part of the abdominal cavity. It appeared by ultrasound as a large nonhomogenous structure with few areas of hypoechogenicity. After surgical removal, the tumor was unevenly large (15.3 cm in diameter) and very hard from outside. After sectioning, the tumor appeared

as having two main parts; a central soft tissues surrounded by a very hard bony structure. Microscopically, the ovary contained insular pattern of round, oval or spindle cells. It had glandular tissues with mucinous secretions and sloughed necrotic cells. Mononuclear cellular infiltration and congestion were apparent. Myxomatous and hyalanized as well as luteinized tumor stroma with congestion and hemorrhage were also noticed. The stroma also contained lipoidal degeneration and steroid tissue. Atretic Graffian follicles with few Schiller-Duval bodies, calcification and hemosiderotic elements were also noticed. The serum estradiol 17-β and progesterone levels just before surgical removal were 38.09 pg/mL and 0.2 ng/mL, respectively.

The ovarian low grade Sertoli cell tumor was palpated transrectally as hard structure extended from the left horn to caudal abdominal cavity. It was difficult to catch the structure completely by hand. By ultrasound, the structure was homogenously echogenic. Removal of the ovary showed a solid fairly round structure. In cut section, two types of tissues could be differentiated, a fairly soft tissue surrounded by a firm one. Microscopically, the Sertoli cell tumor was characterized by complete replacement of the ovarian tissue with aggregated tubules lined by Sertoli type cells and showing foci of congestion or heamorrhage within the tubules or around the tubules. The neoplastic proliferating sertoli cells were arranged in cords or nets with mononuclear cellular infiltration. There was also cystic degeneration of many tubules. These tubules were surrounded with abundant fibrous stroma. There was minimal cytological atypia and very rare mitotic figures, confluent necrosis are seen. No Leydig cells were identified. The serum estradiol-17β, progesterone, and inhibin B levels just before tumor removal were 0.03 pg/mL, 0.03 ng/mL, and 92 ng/mL, respectively.

5.2.2. Treatment strategies

Mares with moderate and severe endometritis were treated intrauterine with one of four treatment protocols: a) Penicillin (Penicillin G Sodium, Biochemie GmbH, Vienna, Austria): 6,000,000 IU/dissolved in 1000 mL 0.9% saline, *n*=30; b) Gentamicin (Gentrin®, Uterine Infusion, Ourofino Animal Health, Cravinhos, Brazil): 300 mg dissolved in 1000 mL water, *n*=25; c) Acriflavine (C14N14CIN3, Fluka chemie EG, Buchs, Switzerland): 1gm dissolved in 1000 mL water, *n*=25; d) Lotagen (metacresolsulphonic acid and formaldehyde, Schering-Plough Animal Health, Segre-France): 1000 mL of 2% concentration, n=19. The treated animals were tested for pregnancy using ultrasound examination 25-30 days after natural mating using fertile stallions. The pregnancy rate was higher in mares infused intrauterine with penicillin (80%) than in those infused with gentamicin (20%), acriflavine (44%), or lotagen (31.6%).

Control of Endometritis in mares:

A normal transient endometritis occurs after mating and often after foaling; this is due to bacterial contamination, but post-coital endometritis may also be a reaction to seminal proteins and spermatozoa. It is considered that endometritis is usually caused by bacteria or yeasts and antibiotic therapy is therefore used. Lavage of the uterus with relatively large volumes of physiological saline may also have beneficial effects, since this will reduce the bacterial population, dilute toxic metabolites and remove cellular debris. However, methods of treatment are many and varied because the relative merits of intrauterine versus other routes of antibiotic treatment have not been extensively

investigated (if i.m. or i.v. therapy is used, general recommendations for dose and length of treatment should be adopted). If intrauterine therapy is used, several aspects should be considered, but there is no universally-accepted method of treatment; the various criteria to be considered are: *Which antibiotic should be used?* Usually there is insufficient time for the results of a sensitivity test to be acted on. However, the most common pathogens are b-haemolytic streptococci, *E. coli*, staphylococci and anaerobes (*Bacteroides fragilis*); antibiotics of choice are therefore penicillin, streptomycin, neomycin, framomycin and nitrofurantoin (for anaerobes). *What volumes of agent should be infused?* Traditionally, the antibiotic is suspended in large (100–500 ml) volumes of water or saline on the assumption that this volume is optimal for filling the uterus; however, the chemical effect of such dilution on antibiotic efficacy is unknown and present evidence suggests that most of the antibiotic introduced in this manner is expelled through the cervix soon after treatment. The use of *small volumes* of antibiotic is appropriate on the assumption that the uterine lumen is only a potential space and therefore easily filled, and little of the agent is thereafter refluxed. Also, if the antibiotic is absorbed from the uterus it should theoretically return to this organ again in therapeutic concentrations in the blood for some time after treatment; *How long to continue with intrauterine treatment?* This is governed by the stage of the cycle. *What stage of the cycle is best for the treatment of endometritis?*

If a mare has no history of chronic endometritis, and is not suffering from pneumovagina, there is little theoretical reason why she should have a positive uterine culture in early estrus; however, these mares do occur, and consideration should be given to the possibility that contamination of the swab has occurred (in this case cytological investigation is valuable). Treatment may then be considered necessary at this stage, before the mare is mated, but since there is no evidence that endometritis is detrimental for sperm transport to the uterine tubes it is probably not necessary to withhold mating.

Treatment of fungal infections can be quite frustrating and the owner should be warned that it can be a prolonged and difficult treatment period. One treatment does use a onetime treatment, although it is somewhat controversial, inconsistently effective, and has somewhat fallen out of favor recently—Lufenuron (Program). This product approved for the treatment of fleas in small animals interferes with chitin production in the cell walls of some yeast as well as the exoskeleton of fleas. The introduction of two packets of the feline 270mg suspension packets in 200 ml of saline has been effective 30–50% of the time in eliminating yeast infections in the author's experience. This should be followed by uterine lavage the following day. One useful broad spectrum treatment for yeast is DMSO. DMSO in a 10% solution is bacteriostatic and fungistatic while in a 30% solution is bactericidal and fungicidal and is irritating but not damaging to the endometrium. Betadine at the rate of 25 ml/liter of saline can be an effective treatment, as can acetic acid (vinegar) at the rate of 20 ml per liter of saline. Beyond these two broad spectrum treatments, therapy should be determined by fungal sensitivity testing, now available from most bacteriology labs. Fluconazole, miconazole, nystatin, and others can often be purchased from compounding pharmacies as suspensions and administered intra-uterine following daily uterine lavage. It is not uncommon for another organism, especially strep to appear as the yeast recedes; for this reason many practitioner include sodium ampicillin or penicillin in the treatment regimes with antifungals. Yeasts and many bacteria produce a biofilm layer that can protect them from disinfectants and many antimicrobials. This can be reduced

before antimicrobial treatment by lavage with 10% DMSO or acetylcysteine (30 ml in 150 ml saline) the day before treatment commences. An acute yeast infection, especially one without uterine fluid accumulation, can sometimes be most effectively treated by a period of reproductive rest. There is no known treatment or prevention for hemorrhagic follicles and no association has been made between the use of ovulatory-inducing agents and hemorrhagic follicles. The recommendations for recurrent anovulatory follicles or unruptured luteinized follicles are to address any uterine inflammatory issues and supplement the mare with Thyroxin. Excessive use of prostaglandins or ovulatory inducing agents should be avoided. Unruptured luteinized follicles are slightly different than hemorrhagic follicles as they actually do start to ovulate but stop short of rupturing into the ovulation fossa. These follicles appear to progress normally right up until ovulation time. They will soften and break through the follicular wall toward the ovulation fossa, ending up with two pockets of fluid in the ovary; one in the original follicle and one in the ovarian stroma. Echogenic fluid will pass rather freely between these two structures for several hours before the fluid starts to clot and eventually luteinizes. These structures routinely respond to prostaglandins and are also more common in older mares. Ovarian hematomas can occur after a normal ovulation and are strictly hemorrhages into an evacuated follicle. These structures are not hormonally active other than the luteal tissue that forms from the original ovulation. They can often be discerned from hemorrhagic follicles by the fact that the hematoma will be clotted within a few hours after it forms, while fluid will remain in the hemorrhagic follicle for days due to the anticoagulant properties of follicular fluid. Ovarian hematomas will generally resolve over a period of 2–3 months. Mares will generally cycle and ovulate normally during this period. This category of mare can be quite frustrating and includes mares that cycle normally, are bred with excellent quality semen in a timely manner, have no observable uterine problems, and repeatedly do not become pregnant. When faced with this type of mare the practitioner should first ascertain that the semen used is resulting in pregnancies at a reasonable rate in other mares. The mare should have a uterine culture and biopsy performed even if there are no other indications to do so. Some mares that present this way are actually hyper-responders that will become pregnant or produce embryos after treatment with immune modulators and corticosteroids. Failure to respond to this treatment in the absence of other abnormalities would suggest an oviductal abnormality.

Two treatment protocols were tested in mares affected with vaginal and/or cervical adhesion: a) Manual dilatation + 5g antibiotic ointment applied on cervix/vagina (Multijet®: 10.000 IU procaine penicillin G, 100 mg streptomycin sulphate, neomycin sulphate, 10 prednsolone, Norbrook, Northern Ireland) + 6,000,000 IU Penicillin /1000 mL saline, infused intrauterine + 20 IU oxytocin IM (Vemedim co, Cantho city, Vietnam), *n*=20; b) Manual dilatation + 5g Multijet+ 6,000,000 IU Penicillin /1000 mL saline, infused intrauterine + 250μg prostaglandin F2α analogue (Estrumate®, Schering-Plough, Morris Ave, Summit, NJ, IM), *n*=16. Both treatment protocols were repeated every 3 days for three successive times. During treatment, the mares were examined for the uterine status using the transrectal ultrasound and for the efficiency of the vagina and cervix using vaginal exploration. When the uterus and cervix/vagina were found sound, the mares were naturally mated, except one that inseminated artificially. Pregnancy diagnosis was performed 25-30 days after mating/insemination. Low pregnancy rates were recorded for both treatment regimes (10 and 18%, respectively) with no significant difference between groups.

Three treatment regimes were tested for mares with ovarian inactivity: a) GnRH (Argoselina®, Buserelin acetate, Laboratories , Santa Fe- Argentina): 0.021 mg (5 mL) IM, once, *n*=15; b) hCG (Pregnyl®,N.V. Organon, Oss, Holland): 1500 IU, IM, once, *n*=10; c) CIDR (Controlled internal drug release, EAZI-BREED CIDR, Pfizer, Auckland, New Zealand): containing 1.9 g progesterone, inserted into the vagina for 14 days, *n*=10. The treated mares of all three groups were observed for estrus. Those showing estrus symptoms were naturally mated and examined for pregnancy using ultrasound 25-30 days after mating. The incidence of estrus induction and pregnancy rate did not differ among treatment regimes (60, 70, and 70%, and 40, 40, and 60%, respectively). However, mares treated with hCG came in estrus significantly later (6.33 ± 0.6 d) than those treated with GnRH (3.66 ± 0.3 d) or CIDR (3.00 ± 0.4 d).

Two treatment regimes were investigated in mares affected with endometrial cysts: a. Sodium chloride (Sigma – Missouri, USA) + penicillin (*n*=22): sodium chloride was dissolved in distilled water in ascending concentration through three successive days (5g/L in the first day, 10g/L in the second day, and 15g/L in the third day). Penicillin (6,000,000 IU) was dissolved in the sodium chloride solution and infused in the uterus; b. Lotagen (n=9): 1000 mL of 4% concentration was infused intrauterine for only one time. Both groups were mated in the next estrus, and examined for pregnancy by ultrasound 25-30 days later. The result showed that infusion of the uterus of mares suffered from endometrial cysts with sodium chloride in ascending concentrations gave significantly greater pregnancy rate (30.1%) than those infused with lotagen solution (11.1%).

Two protocols were compared in mares with pneumovagina: a) Caslik's operation was performed in 12 mares. The operation was carried out on the standing animal restrained in stanchion (Mcllwraith et al., 1998). Sedation was performed by xylazine HCL (0.3 mg/kg body weight, intravenously). Local infiltration anesthesia (2% lidocaine) was done in the ischiorectal region. The tail is bandaged and tied out of the way, and the rectum was manually evacuated before surgery. Just before surgery, the uterus and vagina were flushed with acriflavine 1:1000. The perineum and vulva were surgically prepared. The labia were retracted. An incision was made along the mucocutaneous junction of the labia in a dorsoventral direction and extended cranial along the dorsal commissure of the vestibule to the vicinity of the vaginovestibular junction. The mucosa was then dissected submucosally from the dorsum and dorsolateral aspect of the vestibule, and removed. The incised edge of the mucosa of the right side of the vestibule was then sutured to the incised edge of the left side of the vestibule using a simple continuous pattern of size 0 polydioxanone (PDS). The skin sutures were removed after 10 to 12 days. The suture was removed one month later for mating. The mares were then examined for pregnancy 25-30 days after breeding; b. The other 6 mares with pneumovagina were treated with acriflavine (1:1000) infused intrauterine. These mares were mated in the next cycle and examined for pregnancy one month later. Pregnancy rate tended to be higher (P=0.06) in mares treated with Caslik's operation (41.6%) due to pneumovagina than in those only washed with acriflavine (16.7%).

The uterine tube is extremely important in reproduction, as it is the primary sperm reservoir in the mare, the site of conception, and the home of the embryo for the first 5½ days. Uterine tubes can be obstructed by accumulations of mucus and debris and in the past have been surgically flushed with

some good results. Recently, laparoscopic application of prostaglandin E2 gel to uterine tubes of mares with suspected blockage with extremely good results has been described. Prostaglandin E2 is naturally released by embryos to stimulate contractions of the uterine tube resulting in passage of the embryo to the uterus. Possibly, application of high levels of PGE2 to the uterine tube results in enough contractions and relaxation to remove obstructions. This technique has proven quite valuable with pregnancies resulting in 11 of the first 13 mares treated within 2 cycles following treatment

5.3. Infertility in Cows

5.3.1. Causes

During the period from 2003 to 2006 a total of 2755 cattle were examined, during winter and summer at three districts of Middle Egypt. Based on the owner complains, animals were categorized as anestrum, repeat breeder and those for pregnancy diagnosis. Animals were examined by rectal palpation and by transrectal ultrasonography. The results showed that, ovarian inactivity was the most common cause of anestrum, whereas endometritis was the main frequent finding of repeat breeding. District, season and year affected the incidences of infertility. The pregnancy rate remained constant from 2003 to 2006. The incidence of ovarian inactivity decreased over the same period. In winter, the commonly used feedstuffs would be adequate to supply the animals with needs, but in summer, rations seem to be deficient in many essential nutrients.

Reproductive performance in European and North American dairy herds is declining steadily and is more than ever a highly important limiting factor in efficient herd performance. The following is a brief summary of the more common clinical problems that result in reproductive failure:

Endometritis: bacterial contamination occurs usually after parturition. Endometritis often occurs as a sequel to dystocia and/or retained placenta and may be connected with a decreased rate of involution of the uterus in the postpartum period. Clinical endometritis was identified by the presence of purulent uterine discharge or cervical diameter >7.5 cm after 20 days in milk, or by a mucopurulent discharge after 26 days in milk. Potential treatments can be divided into (1) systemic antibiotic treatment, (2) uterine infusion, and (3) injection of prostaglandin to induce estrus, so that the uterus is cleansed naturally. Uterine infusion and/or prostaglandin injection are treatments of choice. Iodine-based infusions save fallen from favour because they may cause irritation and necrosis. Prostaglandin treatment was more effective than the other treatments in terms of estrus detection efficiency, interval to first service and days open.

Anestrus: nutritional anestrus in the most common problem in beef cows. By feeding cows after they calve, you cannot expect to improve the number cycling. Feeding pre-partum is the best way to assure early return to cyclicity in beef cows. By providing good pre-partum nutrition, you maintain adequate pre-partum condition, so the stress of postpartum lactation produces a shorter duration of negative energy balance. It is quite clear that poor metabolic status and negative energy balance in particular, can compromise a number of aspects of reproductive performance. This is related to the natural metabolic mechanisms that tend to direct nutrients towards milk production at the expense of other

functions such as reproduction. During periods of negative energy balance, which can last up to 20 weeks after calving in very high yielding cows, body tissue is mobilized to make up the shortfall, releasing non-esterified fatty acids (NEFAs) and ketone bodies into the circulation. If cows are over-fat at calving, there is increased mobilization, leading to appetite depression, which in turn increases the magnitude of the negative energy balance. High crude protein levels, typical of present-day cow diets, can lead to an excess of rumen degradable protein in relation to available fermentable metabolizable energy. This leads to an increase in the production of ammonia, which requires energy for its detoxification. This will also worsen the negative energy balance as will high concentrate to forage ratios in the diet, which can lead to acidosis and thus to reduced dry matter intake. The effect of negative energy balance is mediated mainly through a deficiency in IGF-I and insulin levels, which are in turn associated with low concentrations of plasma leptin.

Nutrition: vitamins and minerals are often suspected in infertility and anestrus, especially by feed salesmen, but little hard evidence supports these claims. **Urea** has no effect on reproduction. **Carotene** is needed by the CL. If it is low the cow may have low progesterone and irregular cycles. **Copper** requirements are 10 ppm. Less than that may cause anestrus. A **cobalt** deficiency may cause a delayed first estrus and irregular heats. **Mn** requirements are 40 ppm. Less than this may cause anestrus or irregular heats. **Phosphorus** is hard to separate from energy. It is associated with the seed portion of plant. Cows fed at 66% NRC had no change in pregnancy rates. Cows fed at 50% NRC for 8 months, however had lower pregnancy rates. Experimentally low levels also delayed puberty in heifers.

Silent Heat: silent heat is generally not a problem and usually is manifested by unobserved heats by producer. However, the first postpartum heat is normally silent, because there are no estrogen receptors. This is a result of the low postpartum progesterone. Since the progesterone is needed to induce the estrogen receptors, the estrogen receptors are absent and heat is silent. Synchronization of estrus and using of fixed time AI, introduce a teaser into the herd; and increasing observation time are methods used to control this problem.

Cystic Ovarian Disease (COD), or Follicular Cysts

This is a fluid filled structure on the ovary greater than 2.5 cm in diameter; No corpus luteum present on either ovary; The cyst may persist for more than 10 days or regress and be replaced by another cyst; There are actually two types of cysts, follicular and luteal; A follicular cyst consists of a fluid filled cavity (unovulated follicle) lined by a small layer of thecal cells. The thecal cells produce progesterone, but do not have prostaglandin receptors. If there are no prostaglandin receptors, the normal luteolytic cascade cannot occur, resulting in anestrus. If the granulosa cells persist in an untransformed state (not large luteal cells), the cow will show signs of constant estrus because of the estrogen production. A luteal cyst has a larger thecal (luteal layer) that may have enough large luteal cells to respond to exogenous prostaglandins. A cystic CL is just the cavity in a normal CL that is part of the normal progression of CL development. Both GnRH and human chorionic gonadotrophin (hCG) may be used to treat ovarian cysts, although prostaglandin may be more successful if significant luteinization has already occurred. Progesterone-releasing intravaginal devices have also been used with limited success reported that cows with persistent follicles could be successfully synchronized and inseminated at a fixed time using progesterone, GnRH and PGF2α but showed a limited response

to treatment with GnRH plus PGF2α. Luteinized cysts tend to re-occur after treatment – another reason for culling rather than curing if there is an option. If the condition is associated with nymphomania, the cow can be used usefully as an aid to detecting estrus in her herdmates until she is sold. The incidence of cystic ovaries is considered to be much lower in beef than in dairy cattle.

Delayed ovulation

If a cow ovulates more than 18 hours after end of heat, then ovulation is said to be delayed. This may be diagnosed by palpation and can be treated with GnRH. This only occurs in < 2% of cows and is more likely to be a heat detection problem.

Prolonged luteal function

In the ovarian cycle the corpus luteum normally begins to regress on day 16 or 17. Occasionally and in the absence of pregnancy the corpus luteum may persist for a longer period. Such cases are often accompanied by uterine problems such as endometritis or pyometra and are explained by a failure of the luteolytic mechanism. Also the persistence of a corpus luteum can accompany the presence of a mummified fetus. Furthermore, prolonged luteal function has been recorded in 1.5% of non-pregnant dairy cows in the absence of any detectable uterine abnormality and in these cases the cause is uncertain. Cows that started to cycle less than 25 days after calving were much more likely to develop prolonged luteal function. This may be due to the fact that the first ovulation, or luteinization of a follicle, occurred before the uterus was sufficiently involuted to control luteolysis. Cases of prolonged luteal function are sometimes referred to as 'cystic'. This is incorrect since they may not contain a fluid-filled antrum or cavity and, in any case, corpora lutea of normal life span very often have such a cavity. Prolonged luteal function has also often been diagnosed in cows that have not been observed in estrus. A more probable explanation is that the corpus luteum is normal but that the cow's estrous period was missed. Many cases of prolonged luteal function, especially if they accompany endometritis or pyometra, can cause seriously long delays to conception, but this is not invariably the case. Possible treatments have been discussed for endometritis and pyometra, which is normally associated with persistent luteal function. Whether or not the condition is associated with uterine infection, the best approach is to remove the corpus luteum, which should bring the cow into estrus and thus clean out the uterus. In the past it has been common practice to manually enucleate the corpus luteum per rectum in order to advance the onset of estrus. However, this is not now favored due to the high risk of hemorrhage and/or the formation of fibrous adhesions. Injection of prostaglandin is a more acceptable method.

Pyometra

This condition is due to an accumulation of pus inside the uterus and usually occurs in association with persistence of the corpus luteum. Pyometra can occur as a sequel to chronic endometritis. It may also result from the death of an embryo or fetus with subsequent infection by *T. fetus* or *C. pyogenes*. The uterus is under the influence of progesterone from the corpus luteum and the cervix is closed. Release of PGF2α is prevented due to endometrial damage. The uterine horns are invariably distended, although to a variable degree. However, the condition is seldom accompanied by

systemic illness. The situation may persist undetected for a considerable time since the animal may be thought to be pregnant. Estrogens followed by oxytocin have been used to treat the condition, but $PGF_2\alpha$ is probably more effective.

Campylobacteriosis or vibriosis: the organism *Campylobacter fetus* can cause abortion of the fetus. It has been implicated as a cause of embryo or early fetal loss between 25 and 60 days after natural service, in a herd being monitored by means of milk progesterone profiles. In a smaller proportion of cows, abortion may occur at around five months. In the non-pregnant cow the disease can cause endometritis. The organism can be transmitted between cows by means of the bull that serves them. Infected cows eventually become immune to the disease and will resume normal fertility. The organism is sensitive to antibiotics, so that infected bulls can be treated successfully. The use of AI and its attendant precautions has considerably reduced the incidence of the disease in developed countries in the last few decades.

Trichomoniasis: The *Trichomonas fetus* is a specific pathogen of the bovine prepuce, vagina and uterus. Infection in the female is characterized by irregular estrous cycles, low conception rates, vulval discharge, early abortion and pyometra. The incidence of the disease in many countries has been reduced by the widespread use of AI.

Neospora caninum: infected the reproductive tract and can cause fetal death and later abortions in dairy herds and in beef herds. Fetal neosporosis might be involved in many undiagnosed cases of bovine abortion. Placenta plays a central role in the pathogenesis and epidemiology of the infection. The parasite may attack the fetus directly, but the maternal and fetal inflammatory responses may also be damaging.

Mycotic infections: Fungal infections, particularly those caused by *Aspergillus fumigatus* on mouldy feeds such as poorly made hay and silage, are generally thought to cause abortions fairly late in pregnancy. However, there is some evidence that aflatoxins originating from such moulds can also cause fetal losses at a much earlier stage.

5.4. Infertility in Buffalo-Cows

5.4.1. Causes

A study has been conducted to assess the type and prevalence of abnormalities occurring in the female reproductive organs of 405 buffalo cows slaughtered at Mosul abattoir. Out of the 405 buffalo genital tracts examined, various abnormalities with different degrees of severity were observed in 216 (53.3%) of cases. Twenty two (5.4%) were pregnant and the remaining 41.2% (167/405) were macroscopically normal. The most common abnormalities encountered were endometritis 50 (12.3%), ovarobursal adhesions 26 (6.4%) and hydrosalpinx 20 (4.9%). Other abnormalities recorded were follicular cyst, luteal cyst, cystic corpus luteum, paraovarian cyst, ovarian sarcoma, inactive ovaries, senility anestrous, pyosalpinx, hemosalpinx, obstruction of uterine tube, salpingitis, double uterine tube, hydrometra, mucometra, pyometra, perimetritis, parametritis, uterine edema, perimetrial

adhesions, parametrial adhesions, parauterine abscess and uterine tumor. Histopathological examinations in this study revealed that reproductive organs lesions seem to be an important problem with possible subsequent infertility and sterility in buffalo cows leading to animals slaughtered.

Analysis of the clinical data of 20439 infertile graded female buffaloes of small and marginal farmers in Andhra Pradesh, examined over a period of two years, revealed that 2.50, 73.77 and 23.73 percent of the animals had reproductive disorders which were of anatomical, functional and non-specific infectious nature, respectively. Highest frequencies were of infantile organs, ovarian quiescence and endometritis in each group, which together constituted 79.13 percent. The average gross incidence of various conditions encountered in order of frequency was: ovarian quiescence (56.36%), endometritis (20.68%), silent ovulations (5.3%), anovular heats (4.35%), sub-oestrus (3.48%), luteal persistency (3.37%), genital infantilism (2.09%), vaginitis (o.99%), salpingitis (0.98%), cystic ovarian degeneration (0.91%), bursal adhesions (0.75%), cervicitis (0.3%), hypoplasia of the ovaries and organs (0.24%), paraovarian cysts (0.15%) and hydrosalpinx (0.03%). The incidence of quiescent ovaries followed seasonal trend with significant differences between seasons of the year both in heifers and cows. Between herds and between years, no marked difference in the incidence of different reproductive disorders was observed. It was estimated that 2.93 percent of the animals had disorders which rendered them unfit for breeding and, hence, sterile. Freemartinism was recorded in four cases. The comparative clinical features of freemartinism and cystic ovarian degeneration in buffalo cows were described. The factors predisposing for higher incidence of endometritis in the species were also discussed.

In another study on 135 anestrus buffalo cows, 61.4% had true anestrus with ovarian dysfunction and 33.3% had silent ovulation. In 111 buffalo heifers, 76.6% were in true anestrus and 18.9% had silent ovulation. The duration of anestrus after calving was longer than 6 months in 83% of buffalo cows and 61.5% of the buffalo cows had durations longer than 10 months. The interval between the last breeding and diagnosis of anestrus was more than 5 months in 67.4% of cows and heifers.

In a further study, the most pre-disposing factor for uterine infection was retained placenta (13.3%). The most prevalent bacteria in uterine lumen were *E. coli* (23%), *Archanobacterium pyogenes* (13%) and *Staphylococcus aureus* (10%) in buffaloes with repeat breeding. Vaginal mucus character score was associated with the bacterial growth density score. The difference in PMN was highly significant in animals with repeat breeding than control groups. In addition, PMNs was significantly with the character of vaginal discharge. High level of PMNs observed in buffaloes infected with A. pyogenes. Vaginoscopy examination combined with palpation of uterus increase the accuracy of diagnosing endometritis and cytogenic examination of uterine discharge is more reliable method of establishing the presence or absence of uterine inflammation in buffalo cows. Animals with repeat breeding (endometritis) showed clinical cure and improved pregnancy when treated with oxytetracycline and tylosin. The use of estradiol in repeat breeder cases has no effect in improving neither clinical cure rate nor pregnancy rate. Using manual massage for uterus and ovaries by rectal palpation is a successful method for treating true anestrous in buffaloes.

In a study for the author and his co-workers on 1521 Egyptian buffaloes, anestrous was more common in buffaloes (39.6%) than in cattle (26.8%). The mean duration of anestrous was (187.45 ± 79.8 days). The mean duration of anestrous (from calving until the time of examination) was shorter in cattle (145.98 ± 73.2 days) than in buffaloes (187.45 ± 79.8 days). The incidence of repeat breeding was greater in cattle (20.4%) than in buffaloes (16.6%). Pregnancy diagnosis was carried out more frequently in cattle (52.6%) than in buffaloes (43.8%). Rectal and Ultrasonographic examinations revealed that, ovarian inactivity was the most common cause of anestrous (78.9%), followed by silent heat (16.6%). Miscellaneous causes of anestrous included pyometra/hydrometra/and mummified fetus (2.9%); pregnancy (0.8%); and ovarian cysts (0.8%). Ovarian inactivity was greater in buffaloes than in cattle during summer season and in summer than in winter. Silent heat was common in cattle than in buffaloes, but only in summer. Endometritis was the most frequent cause of repeat breeding (75.6%). The incidence of endometritis was greater in summer than in winter

5.4.2. Treatment strategies

Local antibiotic therapy is the treatment of choice. As endometritis is in high percentage of buffalo cows associated with the presence of persistent luteal tissue, additional treatment with PGF2α is recommended to improve the uterine tonus, evacuate the pathological contents of the uterus and remove the immunosuppressive effect of progesterone.

Treatment of anestrous with prostaglandin $F_2\alpha$ in buffalo cows and heifers with a corpus luteum resulted in higher pregnancy rates within one and two months after treatment as compared with treatment with a vitamin/mineral mixture. Buffalo cows and heifers with inactive ovaries bearing a dominant follicle were also successfully treated with gonadotropin releasing hormone, resulting in higher pregnancy rate within one month after treatment. The use of CIDR 7 days plus i.m. injection of 25 mg of PGF2α in the 6th day plus i.m. injection of 10 μg GnRH in 8th day is an alternative to restart the ovarian activity in buffalo cows.

In conclusion, the predominant cause of anestrus in dairy buffaloes was true anestrus with inactive ovaries, and the duration of anestrus after calving as well as breeding was extremely long. Routine reproductive examination and adequate hormone treatment may improve the reproductive performance of these buffaloes.

Buffalo-cows could be served as early as 30 days post partum using combination of 10 μg GnRH and 25mg of PGF2α.

Treatment with PMSG, GnRH and mineral vitamin mixture gave satisfactory results to manage Egyptian buffaloes suffering from ovarian inactivity.

5.5. Infertility in Ewes and Does

5.5.1 Causes

Abnormalities of the reproductive organs of female sheep were studied by examining 9970 reproductive organs from cull ewes and 23,536 organs from nulliparous sheep (prime lambs) over a period of 12 months in abattoirs in south-west England. Overall, 3.37 per cent of the organs were pregnant (8.11 per cent of cull ewes, and 1.36 per cent of nulliparous sheep), with a peak incidence between September and December. A total of 655 ewes (6.57 per cent) and 459 nulliparous sheep (1.95 per cent) had acquired abnormalities of the reproductive organs. Within these totals, abnormalities of the ovaries accounted for 3.51 per cent (for the ewes) and 10.68 per cent (for the nulliparous sheep) of all the abnormalities, and abnormalities of the ovarian bursa and uterine tube accounted for 42.1 per cent (for the ewes) and 5.23 per cent (for the nulliparous sheep). In addition, uterine lesions (hydrometra and metritis) accounted for 9.92 per cent (for the ewes) and 13.51 per cent (for the nulliparous sheep); lesions of the cervix and vagina (total of 1.44 per cent) and Cysticercus tenuicollis cysts associated with the reproductive organs (total of 3.05 per cent) were less common. Among the ewes the most common ovarian lesions were ovulation tags, and follicular cysts were the most common in nulliparous animals. Lesions such as bursitis, parametritis and abscesses of the reproductive organs were much more common in cull ewes than in nulliparous sheep, probably having arisen from peripartum infections. Hydrosalpinx and hydrometra, in which the intraluminal fluid was clear, were present at relatively high incidence in nulliparous animals, but not in cull ewes. The proportion of organs containing macerated fetal remnants (2.14 per cent of all abnormalities in cull ewes) was lower than expected. It was considered that the functional significance of many of the lesions, such as ovulation tags and C tenuicollis cysts, was likely to be low, although in some cases of the latter calcification of the cyst had occluded the uterine tubes. Other lesions, notably hydrosalpinx, bursitis and metritis were likely to have made the affected animals sterile. The acquired abnormalities were therefore more significant in terms of individual animal infertility than as a major cause of infertility in flocks.

A total of 1000 female goats were randomly sampled for a period of seven months for gross genital defects. Gross genital defects had an overall prevalence of 20.9%, but those that can result into infertility or sterility were detected in 13.6% of the examined goats. The uterus exhibited the highest prevalence of lesions (14.6%) followed by the ovary (8.6%). Uterine tubes and the cervix had rates of lesions of 4.5% and 2.6%, respectively. Cervical lesions included cervicitis (1.4%), haemorrhages (0.3%) and adenomyosis (0.9%). Major uterine lesions included: metritis and endometritis (6.3%), adenomyosis (5.4%), intra-uterine foetal deaths (1.9%), haemorrhages (3.0%), hydrometra (0.5%) and pyometra (0.4%). Others were cystic endometrial hyperplasia (0.2%), perimetritis (0.3%), serosal cysts (0.2%), unilateral caruncular necrosis (0.2%) and melanosis (0.1%). Salpingeal lesions included salpingitis (3.7%) and hydrosalpinx (0.8%). Detected ovarian lesions included mainly tubo-ovarian–bursal adhesions (3.7%), paraovarian cysts (2.2%), cystic corpora lutea (2.2%), cystic graffian follicles (1.1%) and granulosa-thecal cell tumour (0.1%). Others were ovarian quiescence (0.4%) and *Cysticercus tenuicolis* cyst (0.1%). Overall, majority of the lesions were infectious in nature and their prevalence increased with age. Fetal wastage had a prevalence of 38.4%. This study implicated

infections and foetal deaths as the major causes of genital lesions and revealed a higher prevalence of adenomyosis among goats than that reported in literature. Regular herd health investigations should be carried out to determine the extent and nature of the causes of infertility so as to appropriately advice the farmers.

Some pasture legumes contain chemicals called phyto-estrogens, which can affect the reproductive system of sheep. Many subterranean clover varieties and other clovers, such as red clover, contain high levels of the phytoestrogen formononetin. In the long-term, ewes grazing green pastures with high formononetin levels suffer reduced fertility, while short-term effects can include a prolapsed uterus, difficult lambing and a declining lambing percentage.

Endometritis was considered as one of the causes of subfertility in ewes. Most inflammatory lesion of uterus are infectious in origin and result either from ascending infection by organism that normally inhibits the lower genital organs or infectious agents introduced in to the uterine cavity during mating, artificial insemination , or post-partum. The clinical signs of endometritis include a thin, watery, possibly purulent, malodorous vaginal discharge, association with retained fetal membrane, dystocia, retained dead lambs, abortion caused by toxoplasmosis, chlamydiosis, and listeriosis or uterine infection by Trueperella pyogeneserium pyogenes, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella species, Proteus species, Streptococci species and Clostridium species being the isolates. Endometritis in ewes is mostly common in the luteal phase or postpartum and induces embryonic loss, as a result of uterine tissue disruption or direct embryo cytolysis. In addition, the absorption of bacterial components can prevent the growth of Graffian follicles and ovulation. In commercial sheep and goat flocks, diagnosis is seldom made antemortem, and treatment is generally impractical. In animals with a persistent uterine discharge, remnants of a macerated fetus should be considered as a nidus of chronic infection.

Cystic Ovaries (Ovarian cysts) can appear on one or both ovaries and are usually thin-walled, fluid-filled structures. They are the result of follicles that fail to ovulate. This condition seems to be more common in goats than in sheep. Studies indicate that certain superovulation protocols like pregnant mare serum gonadotropin (PMSG), high estrogen intake, and possible phosphorus deficiency, genetic predisposition, and stressful conditions or other health problems during the birthing process or postpartum interval may cause cysts to develop on the ovaries. In goats, genetics seem to play a dominant role. The most obvious signs that a producer may notice relate to the animal's estrous cycles. Animals with cystic ovaries will often have constant or frequent estrus. Other animals may be completely anestrus (not cycle at all). Some females may exhibit male-like behavior.

Hydrometra or pseudopregnancy is characterized by the accumulation of aseptic fluid within the uterus and a persistent corpus luteum. In goats the diagnosis can be easily made by ultrasound. The incidence of hydrometra was investigated in three herds of dairy goats, in two of them during one oestrous season and in the other over three years. The incidence varied between 3.0 per cent and 20.8 per cent with a mean incidence of 9.0 per cent. The incidence in older goats was significantly higher than in yearlings, and the chance of a hydrometra increased with the age of the goats. The finding, during one year, that a pseudopregnancy occurred more often after an induced ovulation, was not repeated in another year.

Infertility may occur due to deficiency of one of the following elements:

Selenium: infertility occurred due to embryonic mortality 3 to 4 weeks after conception. Important times and classes of stock for supplementation are: ewes and rams before mating to improve fertility; mid to late pregnancy for fetus and increase in selenium content in milk; oral and injectable supplementation short acting. Excess supplementation can be toxic.

Cobalt (Vitamin B_{12}): infertility occurred due to increased peri-natal mortality of lambs. Supplementation includes cobalt pasture spray or fertilizer, vitamin B12 injections or cobalt boluses.

Iodine: infertility due to hormonal deficiency and high lamb mortality. Supplementation includes oral dosing with potassium iodide or iodized oil injections.

5.5.2. Treatment strategies

Ovarian cysts: many animals may spontaneously recover from a cystic ovary problem; however, if treatment is required, the most common drugs given are gonadotropin-releasing hormone (GnRH) and human chorionic gonadotropin (hCG). It is recommended that 50-100 micrograms of GnRH or 750-1,000 IU of hCG be given. To prevent the development of cystic ovaries, start by evaluating the animal's feed. Analyze the animal's entire diet for the proper calcium to phosphorus ratio. It should be between 1.5:1 and 2:1 in the total diet. Do not use moldy feed. Toxins found in moldy feed can be high in estrogen. Because injectable estrogen products (estradiol cypionate - ECP) can cause cysts, their use should be monitored by a veterinarian. Genetic predisposition to ovarian cysts is possible; therefore, cull any animals known to produce cystic daughters.

Hydrometra: an intramuscular injection of 5 mg dinoprost caused a discharge of uterine fluid (cloudburst) in 49 cases of hydrometra in goats during the breeding seasons of 1988, 1989 and 1990. A spontaneous estrus after the cloudburst was allowed to occur in 20 of the goats; in nine (45 per cent) of them a hydrometra recurred, three conceived at the first estrus and eight returned to estrus. Estrus was induced in 29 other cases by means of a second intramuscular administration of 5 mg dinoprost, 12 days after the cloudburst. In this group a hydrometra recurred in only one goat, 14 goats (48 per cent) conceived at the first estrus and 14 returned to estrus. Of the animals in which a pseudopregnancy occurred once or more during the same breeding season, 85 per cent became pregnant, compared with 97 per cent of unaffected older goats. The mean number of kids of the goats that became pregnant and kidded after treatment for hydrometra was 2.0 compared with 2.3 for unaffected animals. The results indicate that a single administration of prostaglandin is not a satisfactory therapy for a hydrometra, but that reproductive performance improves when a second injection is given 12 days after the cloudburst.

Endometritis: Systemic antibiotic and prostaglandin F2α injection are helpful in treating such cases. There is no way for local uterine treatment.

Suggested Readings

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APPENDIX: Tables and Figures

Table 5: Causes of infertility in female camels (*n*=3365)

Cause	Incidence
Endometritis	42.5%
Ovarian hydrobursitis	35.6%
Vaginal adhesion	14.8%
Ovarian cysts	1.6%
Ovarian inactivity	1.4%
Anomalies of the genital organs	1.4%
Hydrosalpinx	1.5%
Ovarian tumors	0.5%
Vaginal tumors	0.5%

Table 6: Causes of infertility in mares (*n*=225)

Cause	Incidence
Endometritis	44.4%
Endometrial cyst	11.6%
Narrow cervix /vaginal adhesion / pyometra	16%
Ovarian inactivity	14.7%
Pneumovagina	10.2%
Ovarian tumor	2.7%
Twining	0.4%

Table 7: Causes of infertility in cows (*n*=1720)

Cause	Incidence
Inactive ovaries	44.4%
Endometritis	30%
Silent heat	21.9%
Pyometra / hydrometra / mummified fetus	3%
Ovarian cysts	0.9%

Table 8: Causes of infertility in buffalo-cows (*n*=1033)

Cause	Incidence
Inactive ovaries	68.5%
Endometritis	19.2%
Silent heat	10.8%
Pyometra / hydrometra / mummified fetus	1.2%
Ovarian cysts	0.3%

Table 9: causes of infertility in goats (*n*=307)

Cause	Incidence
Endometritis	20.5%
Uterine adenomyosis	17.6%
Salphingitis	12.1%
Tubo-ovarian–bursal adhesions	12.1%
Uterine hemorrhages	9.8%
Cervicitis / cervical hemorrhages / cervical adenomyosis	8.5%
Intra-uterine fetal deaths	6.2%
Cystic Graffian follicles	6.2%
Hydrosalpinx	2.6%
Hydrometra	1.6%
Pyometra	1.3%
Ovarian quiescence	1.3%
Perimetritis	1%
Cystic endometrial hyperplasia	0.7%
Unilateral caruncular necrosis	0.7%
Melanosis	0.3%
Granulosa-thecal cell tumour	0.3%

Table 10: Types and frequencies of uterine bacterial isolates in relation to various endometritis scores in female camels (*n*=20)

Uterine bacterial isolates	Endometrial Score				
	E0	**E1**	**E2**	**E3**	**Total**
Cory. pyogenses	2x	2x	3x	2x	9x
gram +ve bacilli	1x	1x	2x	1x	5x
Staph. aureus	-	1x	-	1x	2x
E. Coli	-	-	1x	1x	2x
Proteus	-	1x	1x	-	2x
Candida spp.	-	1x	-	1x	2x
Ps. aeruginosa	-	1x	-	-	1x
Kleb. pneumoniae	-	-	1x	-	1x
Strep. spp	1x	-	-	-	1x
B-hemo Strep	-	-	-	1x	1x
Staph. zooepidemicus	-	-	-	1x	1x
Staph. epidermidis	-	-	-	1x	1x
Total	4	7	8	9	28

E0: subclinical endometritis, E1: light endometritis; E2: moderate endometritis; E3: severe endometritis.

Table 11: Types and frequencies of bacterial isolated from uteri of mares affected with endometritis (*n*=20)

Type of Bacteria	Frequency of isolation
E coli	*16x*
Proteus mirobilis	*3x*
Klebsiella oxytoca	*3x*
Streptococcus agalactiae	*3x*
Streptococcus ubris	*2x*
Pseudomonas aeruginosa	*2x*
Neisseria elongata	*2x*
Alloioccus otitis	*2x*
Neisseria cinerea	*1x*
Kocuria kristinae	*1x*
Shewanella puterfaciens	*1x*
Staphylococcus lentus	*1x*
Streptococcus thoraltensis	*1x*
Staphylococcus intermedicus	*1x*
Streptococcus chromogenes	*1x*
Streptococcus equi zooepidemicus	*1x*
Enterococcus faecium	*1x*
Pasterulla canis	*1x*
Enterococcus hirae	*1x*
Gardnella vaginalis	*1x*
Streptococcus parasaguinis	*1X*
Klebsiella pneumoniae	*1x*
Enterococcus gallinarum	*1x*
Sphingomonas paucimobilis	*1x*
Staphylococcus schleiferi	*1x*

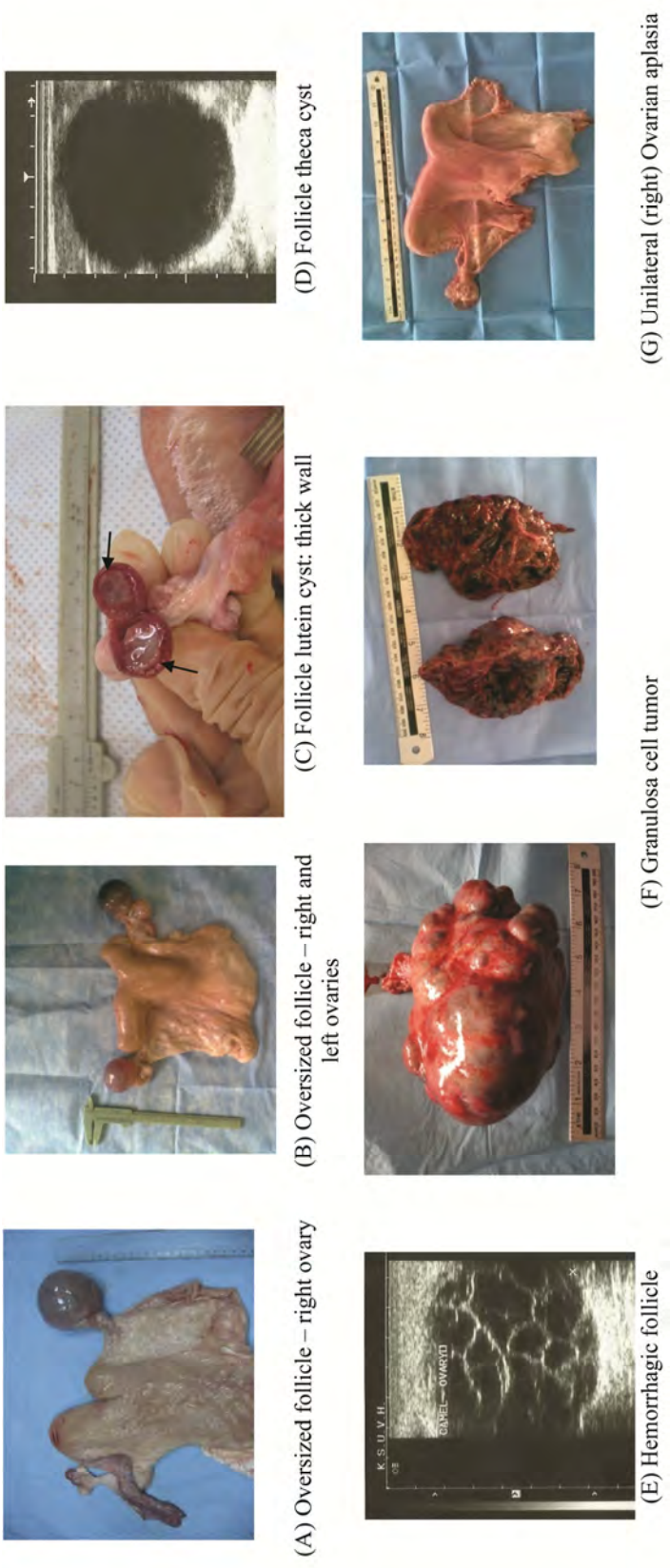
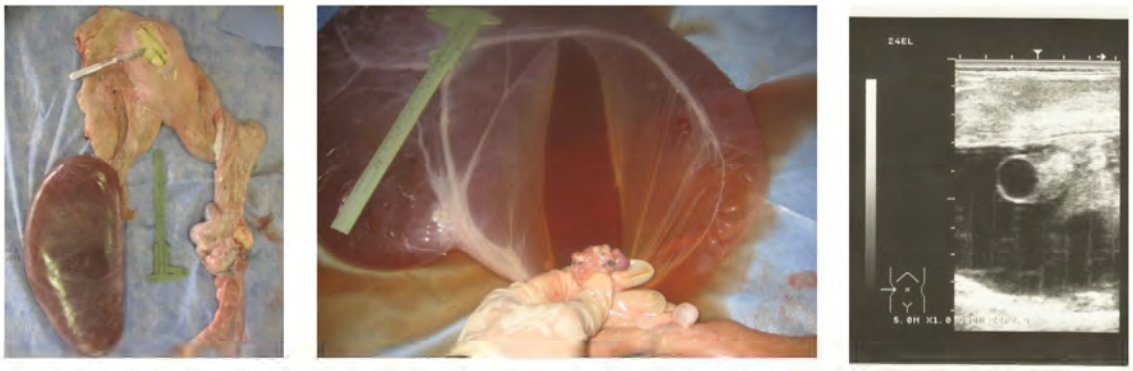
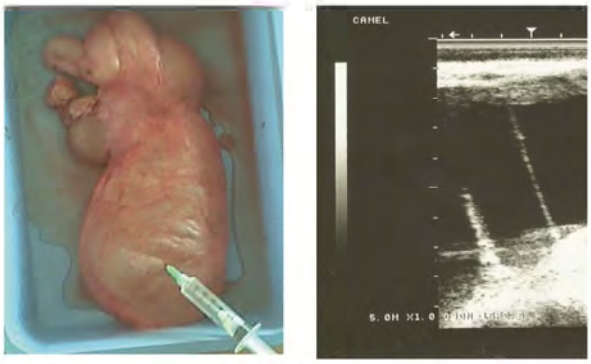


Fig. 16: Diseases of the ovary in female camels (*Makka - Qassim-KSA, 2007-2013*).

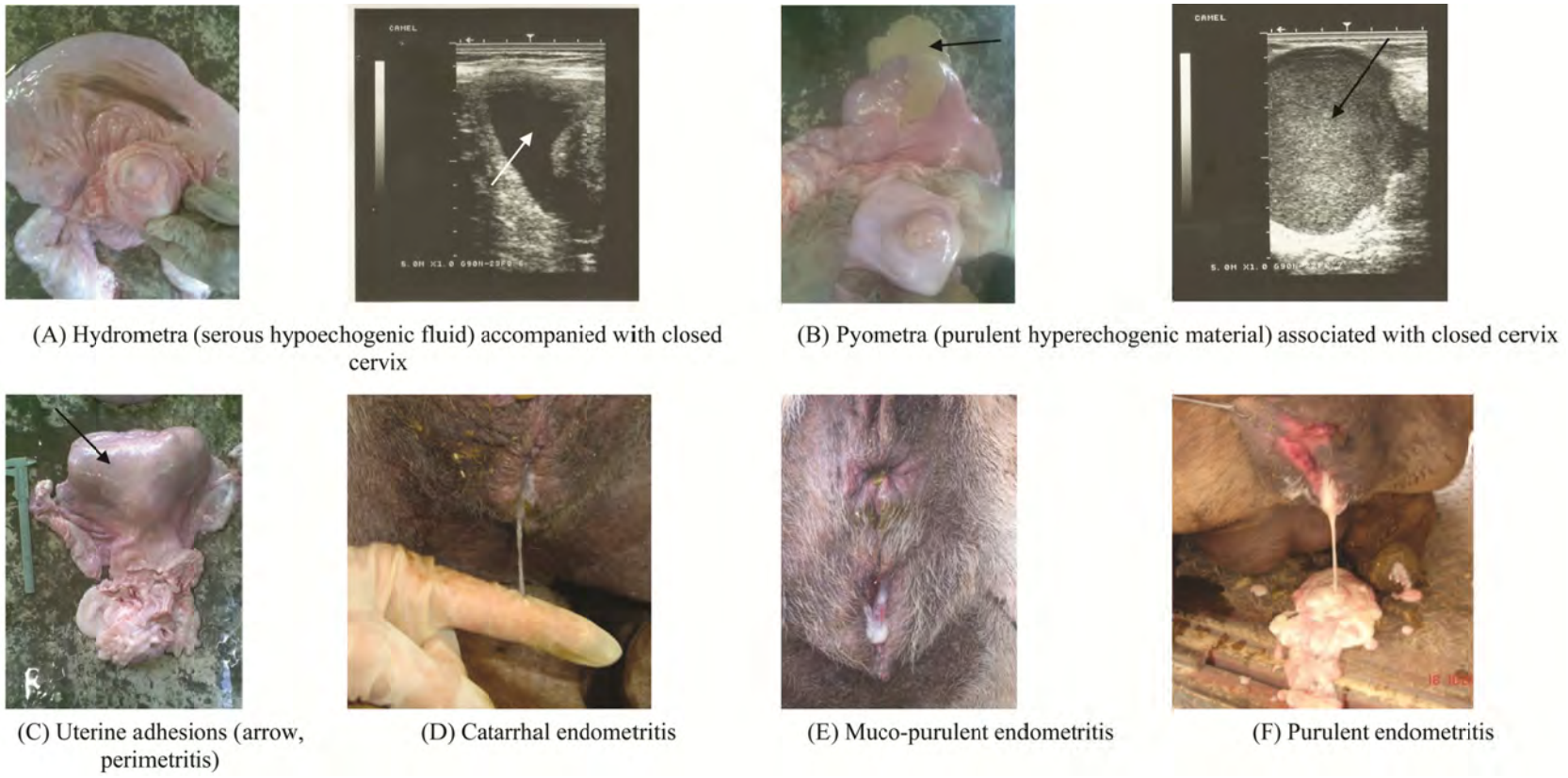


A) Ovarian hydrobursitis: closure of the ovarian bursa around the ovary and accumulation of inflammatory fluid inside it



B) Hydrosalpinx: accumulation of serous fluid insi the uterine tube

Fig. 17: Diseases of the ovarian bursa in female camels (*Makka - Qassim-KSA, 2010-2013).*



(A) Hydrometra (serous hypoechogenic fluid) accompanied with closed cervix

(B) Pyometra (purulent hyperechogenic material) associated with closed cervix

(C) Uterine adhesions (arrow, perimetritis)

(D) Catarrhal endometritis

(E) Muco-purulent endometritis

(F) Purulent endometritis

Fig. 18: Diseases of the uterus in female camels (*Makka – Qassim, KSA, 2007-2010*).

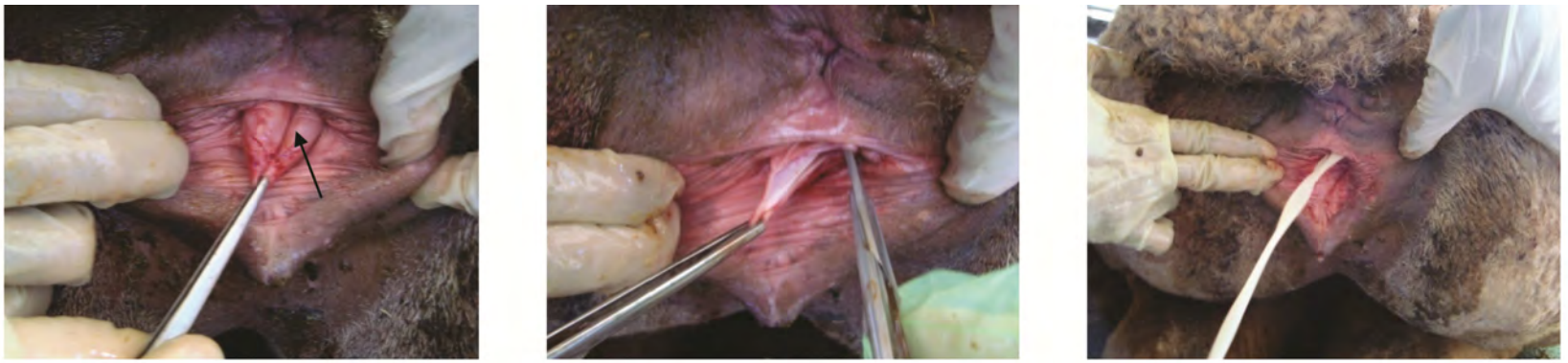
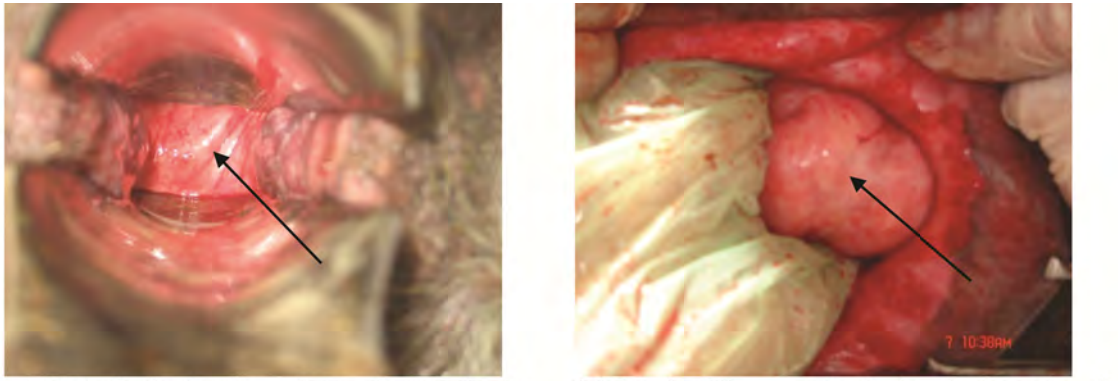


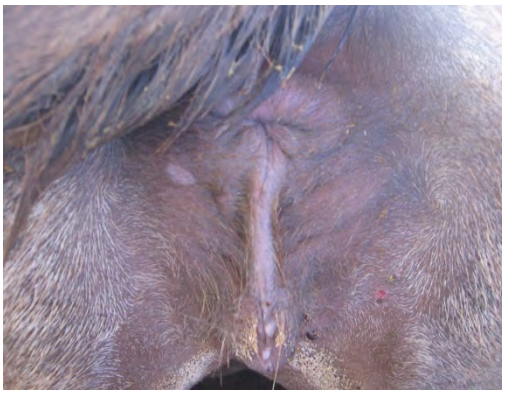
Fig. 19: Persistent hymen (arrow) in a female camel: surgical correction by incising a part of the closed hymen (Qassim-KSA, 2008).



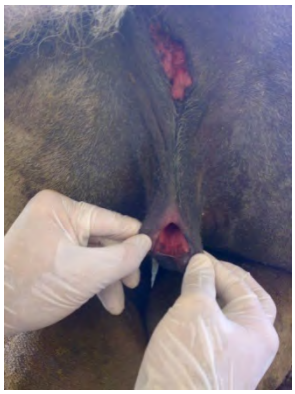
(A) Vaginal adhesions: the vaginal cavity is completely closed by a thick hard tissue

(B) Vaginal lipoma: attached to the lateral wall of the vagina

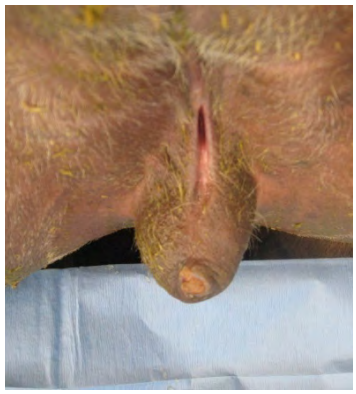
Fig. 20: Diseases of the vagina in female camels (Qassim-KSA, 2009).



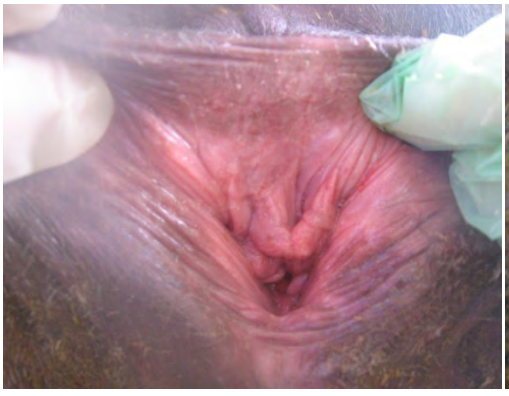
A) Atresia of the vulva: complete closure of the vulva lips with only ventral narrow opening for urination.



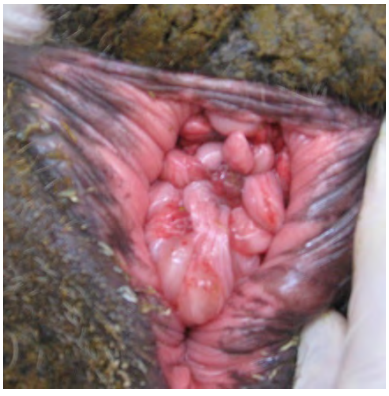
B) Narrow vulval opening



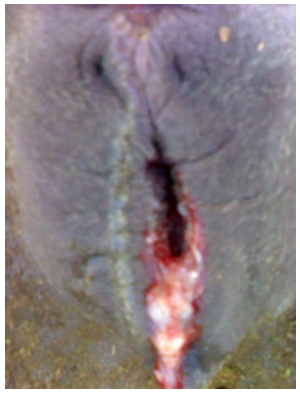
C) Enlarged clitoris and narrow vulva with male behavior (virilism)



D) Protruded fleshy tissues at the dorsum of the vestibulum (called “Said”)

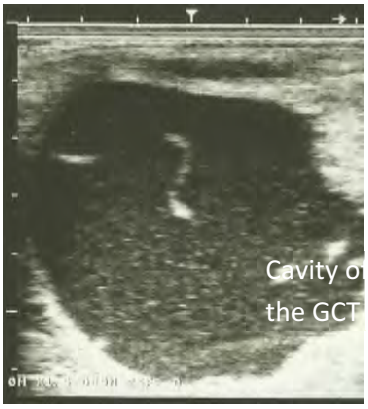


E) Multiple cystic structures in the vestibulum

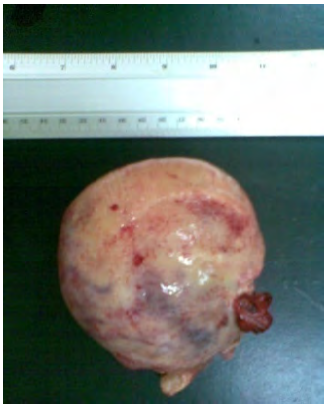


F) Pneumo-vagina with purulent discharges

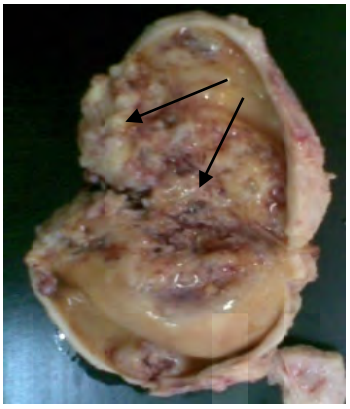
Fig. 21: Diseases of the vulva and vestibulm in female camels (*Qassim-KSA, 2007-2009*).



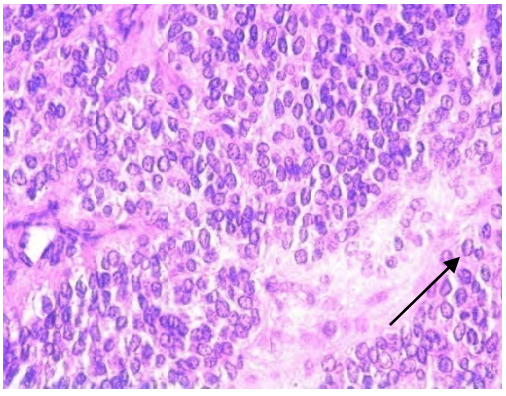
(A) Ultrasonography: cavity of the GCT filled with hypoechogenic fluid with some trabeculae



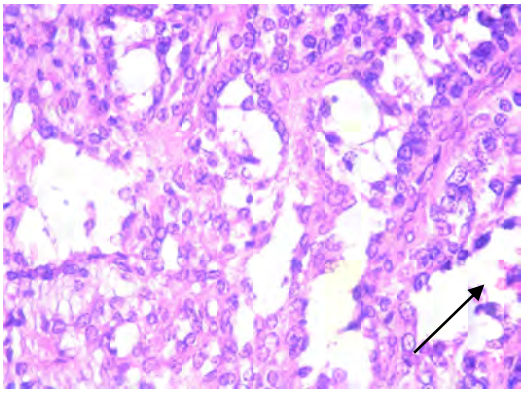
(B) The GCT after surgical removal: smooth round structure.



(C) The GCT after sectioning: large cyst showing mostly a rough inner surface (arrow)

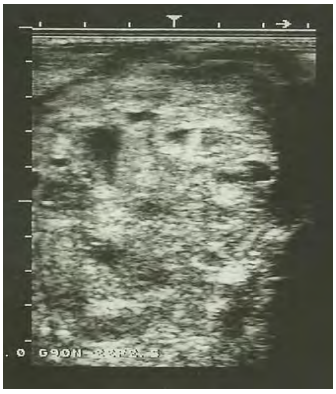


(D) Polygonal neoplastic cells and necrotized eosinophilic stroma (arrow). H&E X400



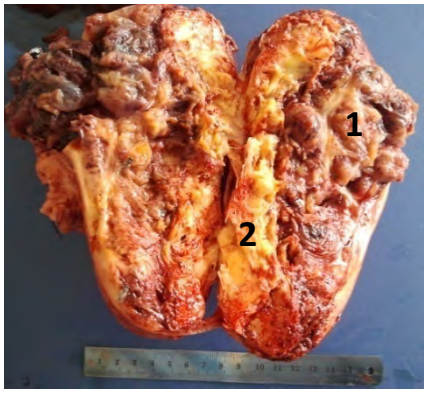
(E) Many cystic spaces in the stroma (arrows). H&E, X400

Fig. 22: Ultrasonography, morphology and histopathology of the granulosa cell tumor (GCT) in a mare (*Qassim-KSA, 2012*).

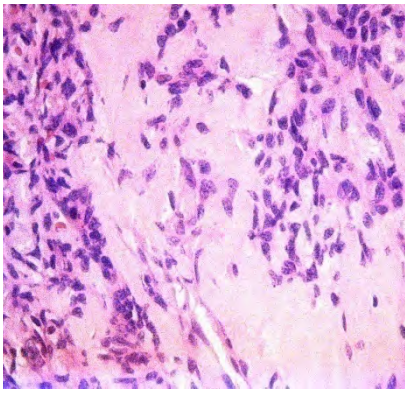


(A) Ultrasonography: unhomogenous echogenic structure

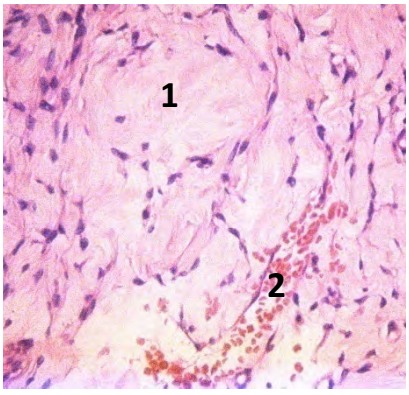
(B) After surgical removal: hard fairly discoidal structure



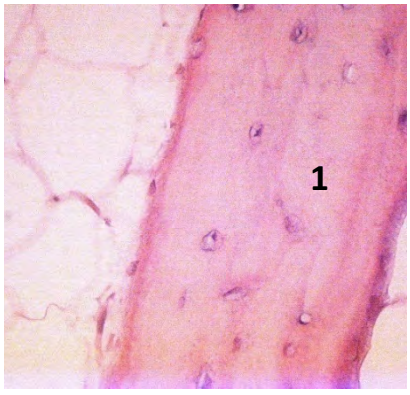
(C) On cross section: soft tissue (1) surrounded by a thick bony layer (2)



(D) Spindle and polymorphic cells. H&E, X400

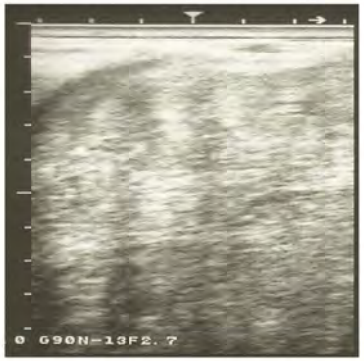


(E) Myxomatous and hyalanized stroma (1) with congestion (2). H&E, X400

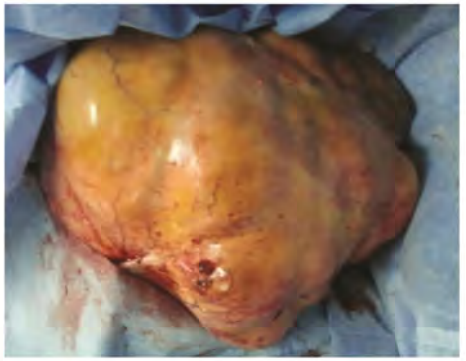


(F) Osteoid tissue (1) detected in the stroma. H&E, X400

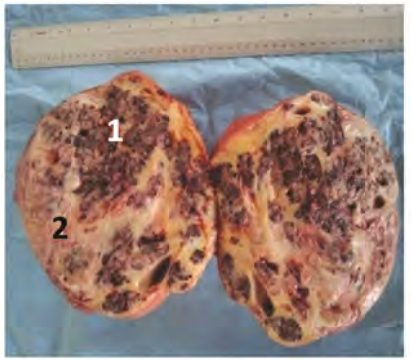
Fig. 23: Ultrasonography, morphology, and histopathology of the mixed germ cell tumor in a mare (*Qassim-KSA, 2012*).



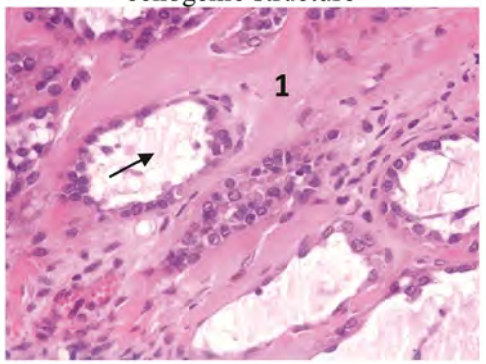
(A)Ultrasonography: homogenous echogenic structure



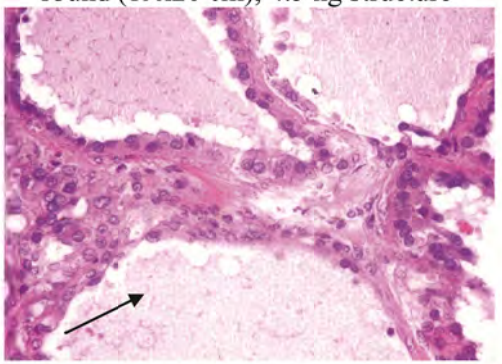
(B) After operation: the ovary was solid and fairly round (19x20 cm), 4.5 kg structure



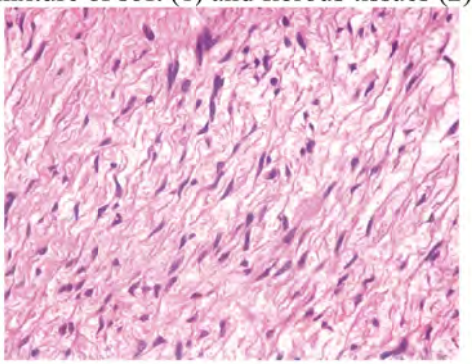
(C) On cross section: the ovary was a mixture of soft (1) and fibrous tissues (2)



(D) Cystic tubules (arrow), hyperleuteinizing stroma (1), with eosinophilic inclusion inside the lumen. H&E, X400

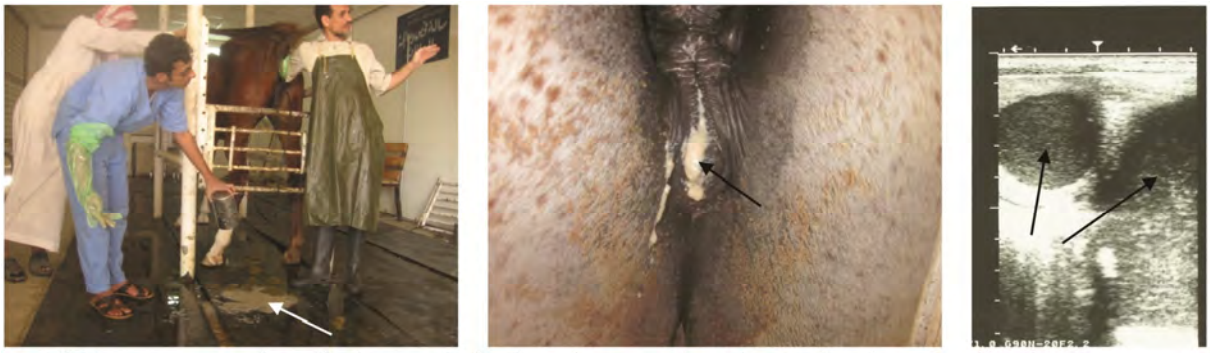


(E) Cystic vacuolated tubules with hypereosinophilic content (arrow), H&E, X400

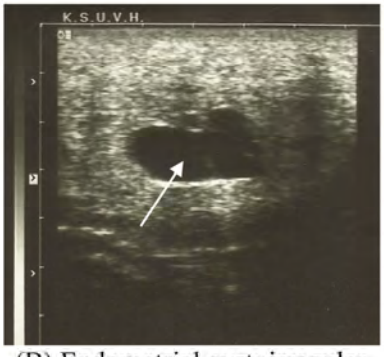


(F) Abundant fibrous stroma with marked sclerotic densely eosinophilic cytoplasm. H&E, X400

Fig. 24: Ultrasonography, morphology, and histopathology of the ovarian Sertoli cell tumor in a mare (*Qassim-KSA, 2013*).

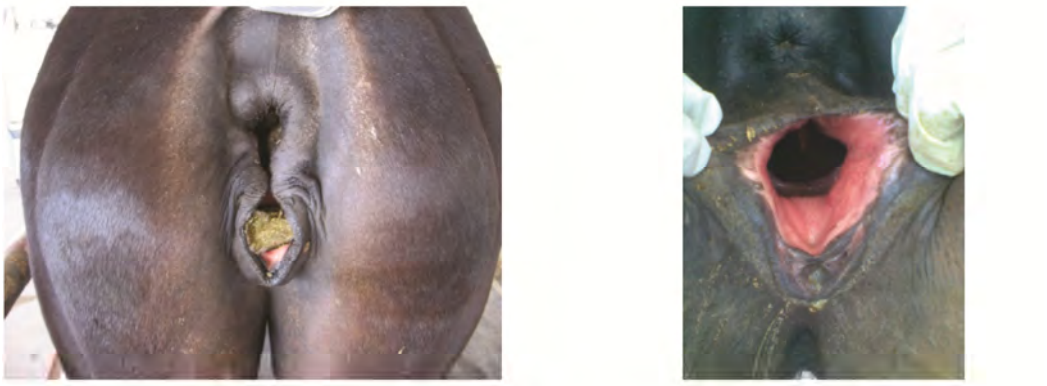


(A) Pyometra: purulent discharges drained on the ground or observed at the vulva lips, it appears as hyperechogenic material inside the uterine cavity

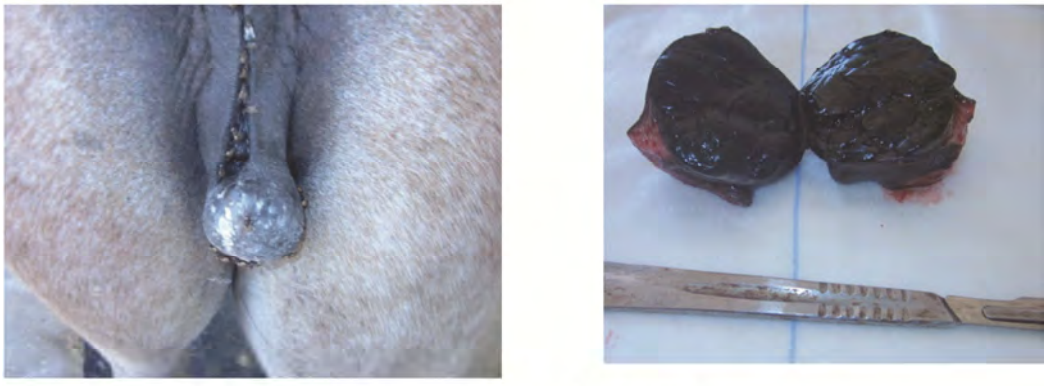


(B) Endometrial cyst: irregular hypoechogenic structure presented eccentric in the uterine lumen

Fig. 25: Diseases of the uterus in mares (*Qassim-KSA, 2007-2013*).

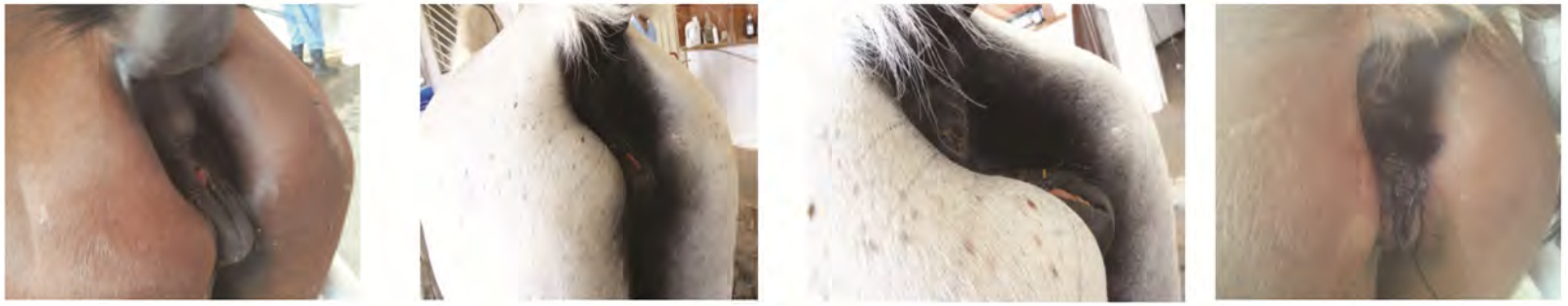


(A) Perineal rupture with accumulation of the fecal matter

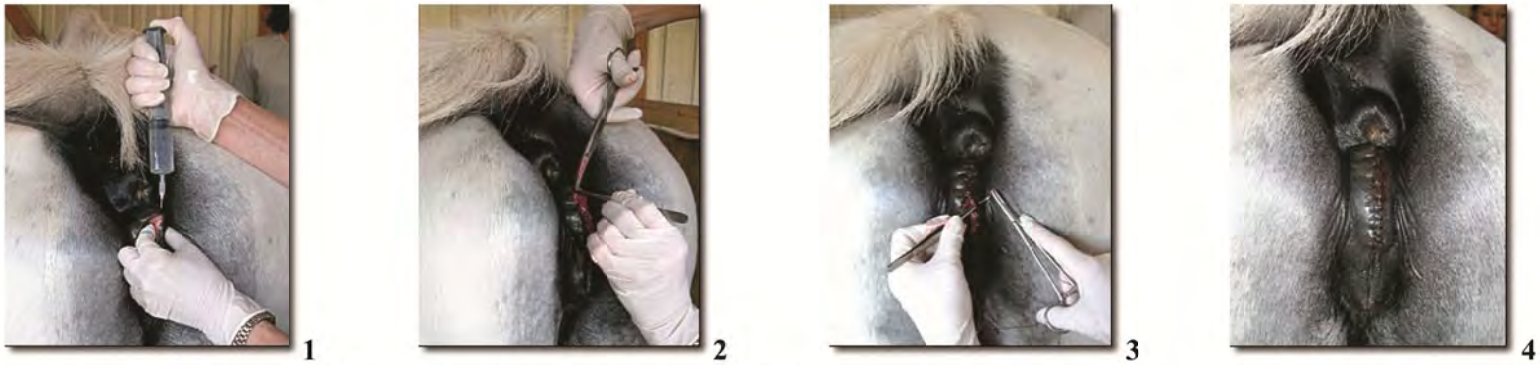


(B) Melanoma of the vulva

Fig. 26: Disease of the vulva in mares (*Qassim-KSA, 2007-2013*).

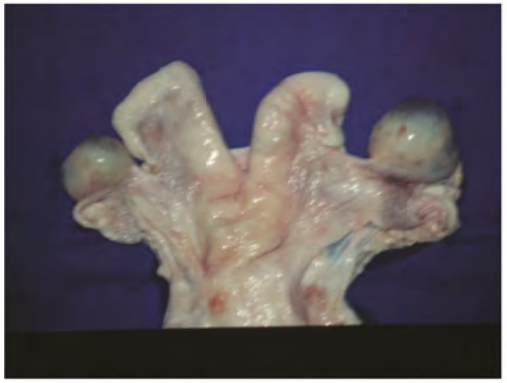


(A) Pneumovagina with different degrees of vaginal inclination in Arab mares.

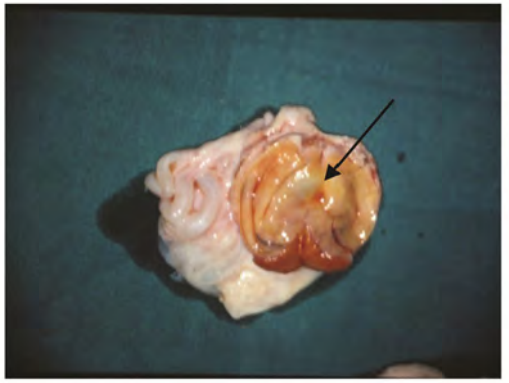


(B) Caslick's operation: 1. Local anaesthesai; 2. Trimming the vulval edges; 3. Suturing the trimmed edge; 4. Sealing the uppert part of the vulva leaving the lower part for urination.

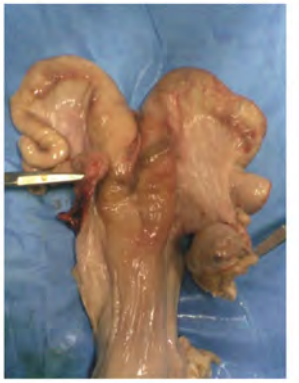
Fig. 27: Pneumovagina (A) and Caslick's operation (B) in mares, (*Qassim-KSA, 2011-2013; www.thehorse.com*).



(A) Follicle theca cysts: both ovaries



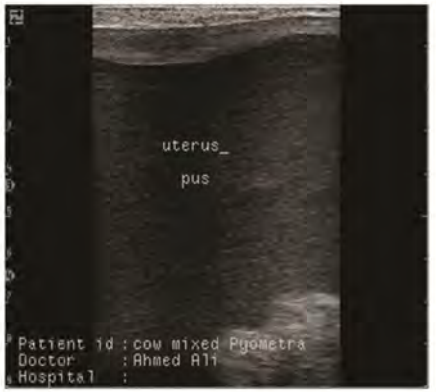
(B) Follicle lutein cyst: thick luteinized wall



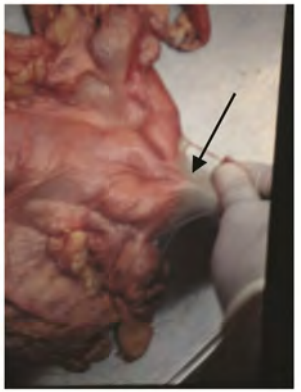
(C) Ovarian adhesion: both ovaries

Fig. 28: Diseases of the ovary in cows (*Assiut-Egypt, Qassim-KSA, 1988-2013).*

(A) Muco-purulent endometritis: vaginal discharge

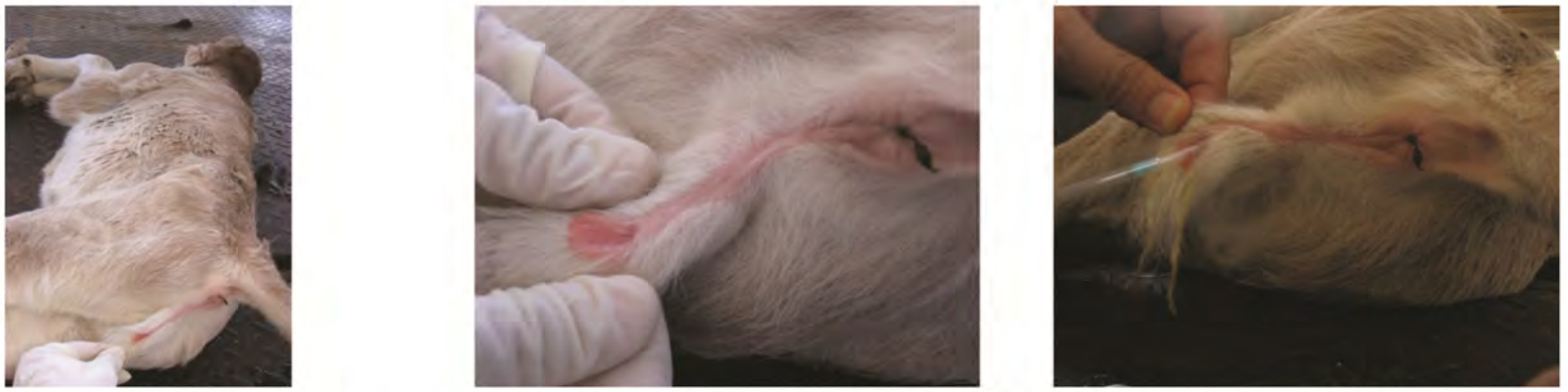


(B) Pyometra by ultrasound: accumulation of hyperechogenic material in the uterus

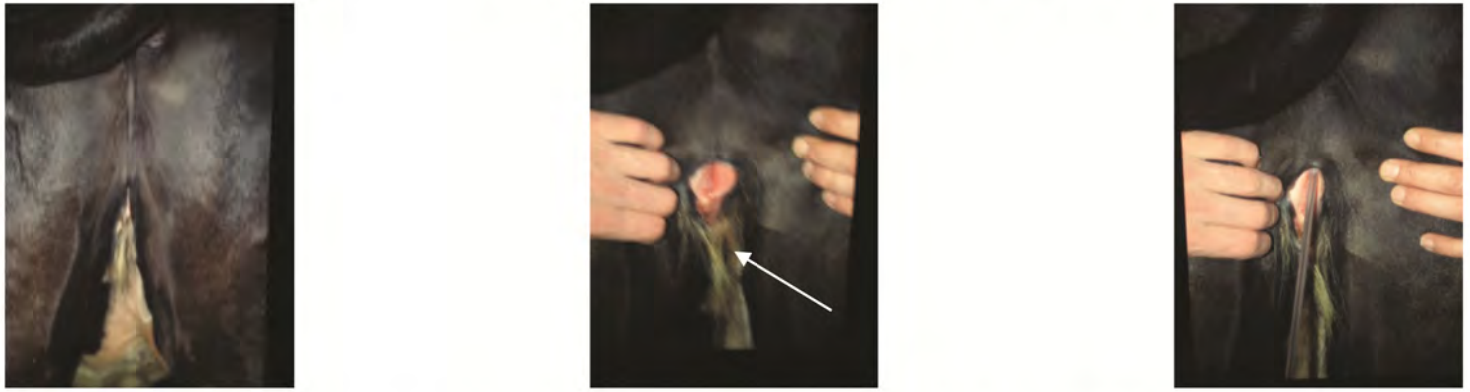


(C) Perimetritis

Fig. 29: Diseases of the uterus in cows (*Assiut-Egypt, 1988-2007*).

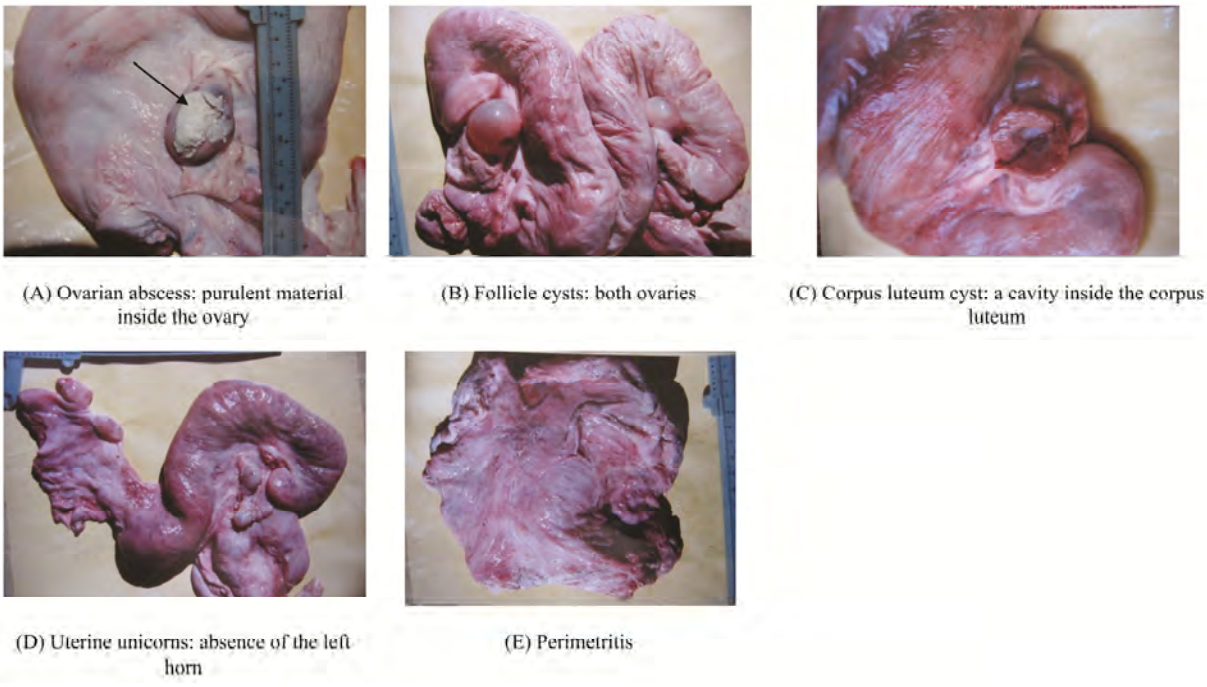


(A) Freemartinism in a calf (6 months): the inseminating catheter did not pass forward in the vaginal cavity



(B) Freemartinism in a heifer (2 years): the vaginal cavity was efficient only for 5cm, a tuft of hair is observed in the ventral commissure, the heifer was masculinized with high hind –limbs and narrow pelvis

Fig. 30: Freemartin in a cow (*Berlin-Germany, Qassim-KSA, 1998, 2010*).



(A) Ovarian abscess: purulent material inside the ovary

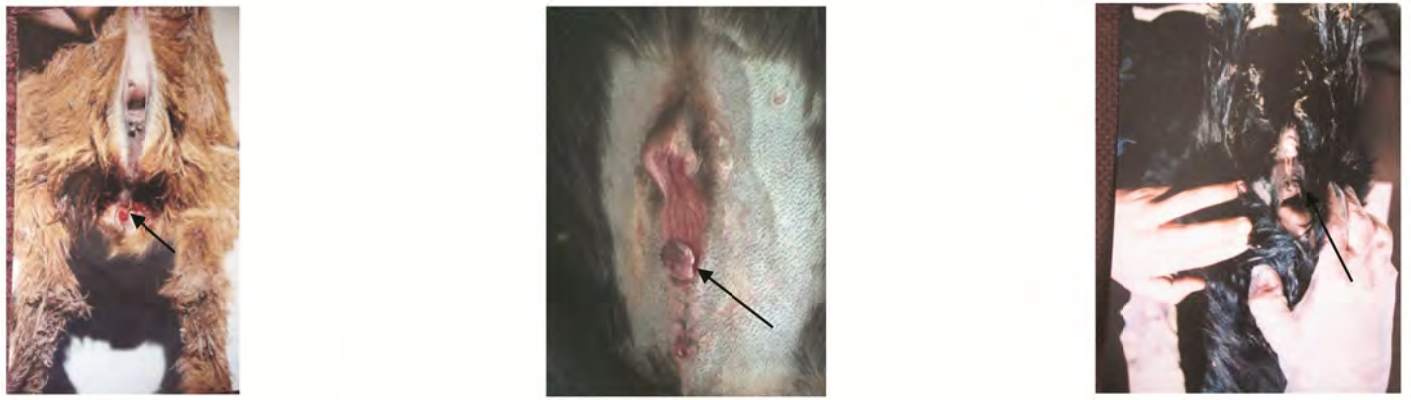
(B) Follicle cysts: both ovaries

(C) Corpus luteum cyst: a cavity inside the corpus luteum

(D) Uterine unicorns: absence of the left horn

(E) Perimetritis

Fig. 31: Diseases of the genital organs in buffalo-cows (*Assiut-Egypt, 1988-2007*).



(A) False hermaphroditism in goats (external genitalia): internally the animal is a female one, externally a glans penis like structure (arrows) is located below the rectum

(B) True hermaphrodite in a goat. The animal had both testes (T) and ovaries (O)

Fig. 32: Hermaphroditism in goats (*Assiut-Egypt – Qassim-KSA, 1988-2013*).

Chapter 6

Pregnancy

The entire process of sexual reproduction is centered on the act of fertilization, which consists essentially of the fusion of two cells, the male sperm and the female egg, to form one single cell, the zygote. Each sperm cell or egg contains only half the normal number of chromosomes or genetic material, so that when fusion occurs the new individual contains the full complement of chromosomes specific for that species. The female egg, which was released from the ovarian follicle surrounded by cumulus cells at the time of ovulation, arrives at the junction one to two days after standing heat, denuded of the cumulus cells and ready for fusion with the sperms. The fertile life span of the ovum is very short (8 to 12 hours). Although the total number of sperm deposited into the female tract measures in the thousands of millions, the number travelling as far as the ampulla is probably not much more than 1000 in any mammal. Some sperms reach the site of fertilization very quickly (15 minutes), but in order to fertilize the egg, sperm must go through changes termed capacitation. The fertile life span of sperm is relatively longer than that of the ovum. The sperm must penetrate two membranes on the egg in order to complete fertilization. The first outer membrane that the sperm must penetrate is called the zona pellucida. Passage of the sperm into the zona pellucida is facilitated by proteolytic enzymes released from the sperm head when its acrosome is lost. The zona pellucida undergoes some sort of change after the passage of a sperm which makes it less easy for subsequent sperm to enter. This is called the zona reaction and is one measure of protection against polyspermy. The last stage of sperm penetration into the egg involves the attachment of the sperm head to the surface of the vitelline membrane. This is a vital period in the fertilization process since it is at this time that activation occurs. Stimulated by the close proximity of the sperm, the egg awakens from its dormancy and development begins. The sperm plasma membrane and vitelline membrane of the egg then rupture and fuse with one another to form a continuous cell membrane over the ovum and outer surface of the sperm. The other defense mechanism against polyspermy is shown by the vitelline membrane and is termed the vitelline block. The fertilizing sperm is actively engulfed by the vitellus; but subsequently, the vitelline surface becomes unresponsive to sperm contact and no further sperm are engulfed. The disadvantage of polyspermy to the organism is that it leads to an abnormal number of chromosomes in the embryo, which is a fatal condition. Once the male and female membranes fuse, the genetic material from each parent is able to join in a process called syngamy to form one new individual. The process of fertilization is now complete and the fertilized ovum undergoes its first cleavage to produce a two celled embryo. Each daughter cell now contains the normal diploid number of chromosomes, half of which have been derived from the egg and half from the sperm. Cleavage of the newly fertilized embryo is simply mitotic divisions of one cell into two cells, two cells into four cells, four cells into eight cells, and so on. The embryo moves from the uterine tube into the uterus at

about the 8-cell stage, usually 3 to 3 1/2 days after ovulation. At this point, the embryo is free-floating within the uterus, and depends upon uterine secretions for nourishment. By the 16- to 32-celled stage, the cells of the embryo are crowded together into a compact group still within the zona pellucida. The embryo is now known as a morulla. Fluid begins to collect between the cells and an inner cavity or blastocyst appears. Once the cavity begins to expand, the embryo is known as a blastocyst. A single layer of large flattened cells, the trophoblast layer, surrounds a knob of smaller cells which lie to one side of the central cavity. Knob or inner cell mass, will give rise to the adult organism while the cells of the trophoblast form the placenta embryonic membranes. Maternal recognition of pregnancy must take place about day 16 to 17, or the uterus will produce prostaglandin F2α which will regress the corpus luteum. If the corpus luteum regresses, circulating progesterone levels will decrease and the embryo cannot implant. It is now believed that embryo produces a protein or hormone-like substances which signal the uterus of its presence and thereby either blocks secretion of uterine prostaglandin F2α or rend the corpus luteum insensitive to its luteolytic action. Significant embryo losses can occur around the time of maternal recognition of pregnancy due to either failure of the embryo to produce the signal or failure of the mother to recognize the signal from the embryo. Progesterone, which is secreted by the corpus luteum of the ovary, acts to decrease muscular activity of the uterus. In addition, progesterone increases stimulates proliferation of the uterine epithelium, and increase the uterine milk secretion. The length of gestation extends from the time of fertilization until birth of the offspring. The fetal and maternal hormone systems interact throughout pregnancy such that pregnancy not only is maintained but that continued development and growth of the fetus is assured. Perhaps the most marked example of the ability of the fetus to regulate the mother's system is its ability to program development of the udder so that milk production is synchronized with parturition.

The Placentation, fetal development, changes in genital organs during pregnancy, and ultrasonographic pregnancy diagnosis are shown in Table (12) and Figs. (33-44).

6.1. Pregnancy in Camels

The gestation lengths for the old and new world camelids are 13 and 11 months, respectively. However, longer and shorter gestation periods have been reported. This variation in gestation length could be due to the method of husbandry, number of matings over the entire period of estrous, number of pregnancies, sex of the fetus, breed or season of conception. Embryos resulting from fertilized ova in the right horn must have migrated to the left horn for unknown reasons. The embryotoxic effect associated with the right uterine horn, an alternative that involves prostaglandin-F2α- mediated luteolysis has been suggested. However, right horn pregnancy has been recorded in dromedary camels.

6.1.1. Development of the conceptus

At ovulation the ovum or egg is collected by the fundibular end of the uterine tube or uterine tube. It is transported down the uterine tube towards the uterus possibly by a combination of cilial (hair-like) action and muscular contractions. Transport through the uterine tube appears to be under the control of ovarian steroid hormones since estrogens reduce and progesterone increases the speed of

passage of ova through the uterine tubes. Fertilization normally occurs in the ampulla section of the uterine tube close to the junction with the isthmus. The embryo usually enters the uterus between days 6 and 7 after ovulation in the dromedary. On Day 6 after ovulation the camel embryo is a spherical early blastocyst still encased within its zona pellucida. The blastocoele had started to develop and the ICM is clearly visible at one pole of the embryo. By Day 8 the embryo has increased in size and has now become a fully expanded blastocyst that has hatched from its zona pellucida. At Day 10 the embryo is just starting to elongate so it now has the outline shape of a rugby football of about 4 mm length. By Day 12 it has further elongated to approximately 20 mm in length.

Camelids, although closely allied to ruminants, have a "diffuse epitheliochorial" placenta.

By Day 14, the majority of the trophoblast had become closely opposed to the luminal epithelium of the endometrium to form the start of an epitheliochorial placenta. By Day 25 microvilli on the surface of the trophoblast cells interdigitate with those on the surface of the underlying luminal epithelial cells to form a well developed microvillar junction between fetal and maternal tissues. By Day 35, the fetus can be easily seen situated in the middle of the left horn and its head, neck and limb buds are easily discernible. Histologically, large multinucleate giant trophoblast cells had developed at frequent but irregular intervals along the trophoblast. The allantochorion now covers the entire luminal surface of the left horn and body of the uterus but it has only reached halfway up the right horn. By Day 56, the foetus now has a more finely defined head, neck, limbs and tail and the allantochorion now fills both the right and left horns. Histologically, numerous large, multi-nucleate syncytium-like cells are present in the trophoblast layer.

During the second half of gestation, there is a little real change in the trophoblast during the rest of gestation. The large multi-nucleate cells are present throughout pregnancy. However, the chorionic villi become more extensively branched and very dense intra-epithelial and sub-epithelial networks exist both in the chorion and endometrium, so the maternal and foetal capillaries are in very close contact with each other.

In a study for the author and his co-workers, serial ultrasonographic examinations were carried out on seven pregnant dromedary camels.

6.1.2. Endocrine control of pregnancy

Progesterone: Studies on progesterone concentrations during pregnancy in the camelidae confirm that these species depend on ovarian progesterone throughout their pregnancy. Ablation of the CL-bearing ovary or administration of PGF2α or its analogue causes abortion or premature parturition at all stages of pregnancy, thus it would seem likely that the placenta either fails to secrete progesterone at all, or it does so in amounts insufficient to maintain pregnancy without help from the ovaries. In the mated dromedary, serum progesterone concentrations increase from day 3 after ovulation to concentrations of around 3.4 ng/ml by day 8. If the camel is not pregnant, concentrations rapidly return to basal levels of <1 ng/ml by days 10 - 12, however, if she is pregnant the progesterone concentrations are maintained between 3 and 5 ng/ml for the first 90 - 100 days of gestation. According to some studies, progesterone levels then decrease slightly to 2 - 4 ng/ml where they

remained until day 300. A further slight decrease then occurs over the next 70 - 80 days followed by a rapid drop to values of <1 ng/ml on the day before, or the day of parturition. Other studies have shown there to be a gradual decrease in progesterone concentration from 5 months of gestation until parturition.

The measurement of progesterone concentration in peripheral blood can thus be invaluable in the early detection of pregnancy. If a blood sample is taken between days 12 - 15 and the value is still high (i.e. >1.0 ng/ml) this would indicate that the camel is possibly pregnant. If the value has dropped to <1.0 ng/ml then the camel is definitely not pregnant.

Estradiol-17β: Serum oestradiol-17β concentrations show a first definite increase around day 20 - 25 after ovulation and continue to rise until concentrations of around 100 pg/ml are reached between days 60 - 70. This increase in rate of secretion of estrogen could be ovarian or placental in origin. The latter seems more likely as we have shown that extra embryonic membranes of the camel conceptus possess considerable aromatizing capacity from as early as day 10 after ovulation. The timing of the estrogen increase in the final 70 - 80 days in the pregnant dromedary coincides with the important period of increase in fetal weight and fetal fluid volume between 9 and 12.5 months.

Estrone sulphate: In dromedaries the oestrogen sulphate concentrations show two definite peaks of about 10 ng/ml in early gestation. The first peak occurs around day 26 and the second around day 70.

13, 14 dihydro-15- keto prostaglandin F2α (PGFM): Secretion of prostaglandin F2α in the dromedary, as revealed by measurement of its metabolite PGFM, remains between approximately 100 - 200 pg/ml during the first 320 days of gestation, thereafter over the next 50 days it rises sharply to around 1000 pg/ml, before a further explosive increase to peak values of 2000 pg/ml on the day of calving.

Thyroid Hormones: Thyroid hormones play an important role in modulating metabolic activity, growth and differentiation of vital organs. The average peripheral concentrations of T4 (thyroxine) and T3 (Triiodothyronine) in pregnant dromedaries varies from 76 to 116 ng/ml and from 0.7 to 1.3 ng/ml respectively.

Relaxin: Relaxin is probably secreted by the feto-placental unit and is implicated in the growth of the uterus during pregnancy and relaxation of the ligaments and cervix at the end of pregnancy.

Antiluteolytic substance: The camel is basically a ruminant but it has an epithelial-chorial placenta like a horse and pig and it is an induced ovulator in which the CL that develops after a sterile mating has a lifespan of only 8 - 10 days. Hence, during normal pregnancy the camel conceptus must pass its antiluteolytic signal to the endometrium by day 8 or 9. Until recently no one had tried to indentify this maternal recognition pregnancy signal in camelids, Some experiments were carried out to investigate the possible roles of either foetal oestrogens or interferon-like proteins in this process. Results of incubation experiments of known weights of embryonic tissues showed convincingly that the young camel conceptus does not synthesize any interferon-like proteins similar to ovine IFN. In contrast however, further incubation studies of embryonic tissue with tritiated (i.e. radioactively labeled with ^{3}H) estrogen precursors (i.e. androstenedione) showed even more clearly that they produce strikingly

large amounts of both estradiol -17β and estrone from as early as day 10 after ovulation but concentrations of both oestradiol-17β and estrone declines from day 22 onwards and is negligible by day 60. This onset of estrogen synthesizing ability by embryonic tissues coincides well with the observed time of luteolysis following a sterile mating, thereby prompting the suggestion that these fetal estrogens may form an important component of the vital maternal recognition of pregnancy signal that must be transmitted by the embryo to maintain luteal function.

6.1.3. Pregnancy diagnosis

External signs: refuse mating and tail "cocking" are possible to detect pregnancy in camels as early as two weeks after mating. The pregnant female erects and coils tail when approached by a male camel. However, these symptoms have been noted in female camels having closed/adhered cervix accompanied with or without uterine distension with fluid or pus.

Rectal Palpation: The membrane slip test, described in cattle pregnancy diagnosis, is not possible in camelidae because of the diffuse type of placentation. Therefore positive pregnancy diagnosis can only be achieved if the CL and fetus are palpated. The earliest sign of pregnancy is the persistence of the CL which continues to grow until day 35 of pregnancy. It is usually soft, flabby and spherical in shape, measuring about 25 mm in diameter, but becomes out of reach after about 90 days. It is not until about day 45 that uterine changes due to pregnancy can be detected by rectal palpation and the first sign is an increase in the diameter of the left horn. However it is not until approximately the third month of pregnancy that the gravid horn feels obviously bigger and softer than the non-gravid horn and the uterus becomes more abdominal as the amount of fetal fluid increases. The cervix is pulled forward and lies just at the brim of the pelvis at 4 months, and by the fifth month the uterus is completely in an abdominal position with a small degree of fluctuation, but the fetus is not always palpable. From the 6 month onwards the fetus can be palpated, first by ballottement, and then the head and legs become easily palpable as the fetus starts its ascent. Precise estimation of the stage of pregnancy by rectal palpation in the dromedary is not possible beyond 3 months because of the absence of structures such as cotyledons and difficulty in reaching the fetus in this species.

Ultrasonography: in a study conducted by the author and his co-workers, a total of 329 examinations were conducted between the 2nd and the 54th weeks of pregnancy. Intrauterine fluid accumulation was detected between the 2nd and 3rd weeks of pregnancy. The embryo proper was noticed between the 3rd and 4th weeks. Organization of the embryo was first observed between the 6th and 7th weeks. Ossification was first detected between the 7th and 9th weeks. The accessibility during the total gestational period was 35/329 (10.6%) for crown-rump length, 35/329 (10.6%) for biparietal diameter, 42/329 (12.8%) for abdominal diameter, 42/329 (12.8%) for ruminal length, and 126/329 (38.3%) for eyeball diameter. A high correlation was found between gestational age and each of the studied parameters. The highest correlation was found with the crown-rump length and the biparietal diameter during the 1st trimester and with the eyeball diameter during the 3rd trimester of pregnancy. The overall accuracy of the ultrasonic prenatal fetal sex assessment was 91.7%. The best window was found during the 11th week of pregnancy. It was concluded that sonographic fetometry can be useful for the evaluation of fetal development, the estimation of gestational age and the prediction of prenatal fetal sex in camels.

6.2. Pregnancy in Mares

6.2.1. Development of the conceptus

Pregnancy length is approximately 330–345 days in the mare, but is very variable, with extremes of 310–370 days or even longer occurring not infrequently. The median pregnancy duration in Arabia mares is 335 days (range 320-360 days). After fertilization, the conceptus reaches the uterus about 5–6 days later. The conceptus is mobile in the uterine horns and body for up to 16 days. The mobility phase is important for the maternal recognition of pregnancy. The conceptus becomes lodged at the base of one of the uterine horns. Lodging (fixation) of the conceptus occurs on day 16. The embryo proper develops on the ventral aspect of the conceptus. Development of early allantois between embryo and trophoblast raises the embryo dorsally. The embryo can now be identified using ultrasound. As the yolk sac (which is dorsal to the embryo) becomes smaller and the allantois becomes larger, the embryo moves further dorsally within the conceptus. The mesoderm surrounding the yolk sac carries blood vessels. The umbilicus will be attached dorsally at the base of one uterine horn. After about 35 days, most organogenesis is completed and the embryo is called a fetus. The fetus remains in the uterine horn until about 70–80 days, but is then usually in the uterine body until 6–7 months. By now, the fetus is becoming too large be held by the uterine body alone, and its hindquarters begin to occupy one horn, usually the original pregnant horn. After this time the fetus cannot change its presentation. Until just before the second stage of parturition, the fetus usually lies in a ventral or lateral position, with limbs, head and neck flexed.

The uterus becomes progressively more turgid from about 15 days to 21 days post ovulation. The conceptual swelling protrudes at the base of one of the uterine horns and can be detected by palpation. This swelling is 3–5cm in diameter and it bulges ventrally. The uterine wall over the conceptus is thin, but the persistent tone in the adjacent uterus keeps the conceptus in place after day 16. As the conceptus grows, the swelling becomes larger but remains roughly spherical. By 60 days, the swelling is about 12 cm in diameter and fills the pregnant horn. The body and non-pregnant horn are still tonic. After 60 days the swelling usually becomes less tense and starts to involve the body and eventually the non-pregnant horn. By 90 days, the whole uterus is filled with fluid. Further distension of the uterus causes the ventral surface of the uterine body to lie against the ventral body wall. The dorsal surface of the uterus is suspended by the broad ligaments. Distinction between body and horns becomes less obvious.

On day 36, endometrial cups start to develop in a ring round the equator of the conceptus. The endometrial cups produce equine chorionic gonadotrophin (eCG) (previously termed pregnant mare serum gonadotrophin (PMSG). As the conceptus becomes larger, cups are adjacent to the dorsal aspect. On 100–150 days, the cups become necrotic and slough off the surface of the endometrium, and come to lie between it and the allantochorion. The *hippomane* is a soft calculus of cellular and inorganic debris which forms in the allantoic cavity. Occasionally there are accessory small hippomanes either free in the fluid or attached to the chorioallantoid membrane, particularly on the chorioallantoic pouches (wrongly called ‘false hippomanes’).

6.2.2. Endocrinology of pregnancy

Early pregnancy (the first 14 days) is similar to the non-pregnant luteal phase. However, in the non-pregnant mare the endometrium secretes prostaglandin on approximately day 15 after ovulation. This causes regression of the corpus luteum and the return to estrus. In the pregnant mare the conceptus produces a signal (called: *maternal recognition of pregnancy)* which prevents the production of prostaglandin. The early mobility of the embryonic vesicle is important to ensure that all areas of the endometrium receive the signal. The most important time is 14–15 days after ovulation. Impairment of the mobility of the conceptus at this time is most likely to result in an incomplete signal and the production of prostaglandin from some areas of the endometrium.

The corpus luteum that forms after ovulation is called the primary corpus luteum. Progesterone production from the primary CL starts to decline from day 25 onwards. Secretion of eCG from the endometrial cups is first detectable between days 35 to 42 after ovulation, reaching peak concentrations at approximately day 60. It is thought that eCG is responsible for maintaining progesterone production from the primary CL, but in addition it is responsible in part for the development of secondary, also called accessory or supplementary CLs. These structures are formed from either ovulation or luteinization of follicles that are present in the ovaries at this time.

Progesterone: there are significant variations in progesterone concentration throughout pregnancy in the mare. Progesterone concentrations (from the primary CL) usually decrease slightly at day 14–16 post ovulation, but are rarely less than 1ng/ml. Progesterone also starts to decline approaching day 30 after ovulation. From approximately day 40, concentrations increase due to stimulation of the primary CL by eCG and development of secondary CLs under the action of eCG. At this time ovaries are usually large. Progesterone produced by the primary and secondary CLs maintains the pregnancy for the first five months of gestation. eCG concentrations start to decline after day 70, and progesterone concentrations follow a similar trend. Peak ovarian progesterone production generally occurs at approximately day 80 after ovulation. All CLs have degenerated by 200 days after ovulation; after this time ovaries are very small. The feto-placental unit also synthesizes progesterone, which appears in plasma at approximately day 30 and increases gradually until day 300. At approximately day 200 there is therefore, a transition from ovarian progesterone to placental progesterone dependence. Placental progesterone acts locally, so blood concentrations are low after five months of gestation. Progesterone concentrations increase just before parturition.

Equine chorionic gonadotrophin (eCG) or PMSG: eCG is produced by the endometrial cups. eCG concentrations increase in plasma from approximately day 40. Peak concentrations are reached at approximately day 60. At 100–150 days the cups become necrotic and slough off. eCG concentrations are normally basal by day 120.

Estrogen: there are several sources of estrogen in the pregnant mare. Estrogens produced by the early conceptus as early as day 12. These estrogens are locally produced and do not increase circulating concentrations. Estrogens produced by the ovary, increase at approximately the time that eCG is produced from the endometrial cups. Additionally, estrogens produced by the feto-placental unit, increase after day 60. The large amount of estrogen in late pregnancy is produced by the fetal gonads.

In general, until approximately day 80, plasma estrogen concentrations are low; thereafter vast quantities of equilin, equilenin, estrone and 17-*B* estradiol may be detected. Both blood and urine concentrations of these estrogens remain high until 300 days, after which they decline to parturition. Estrone is conjugated to estrone sulphate in the fetal liver; the amount of this hormone in the maternal circulation is an indication of fetal 'well-being'.

Luteinizing hormone: LH concentrations are low throughout gestation.

Follicle stimulating hormone: FSH is released episodically up to 40 days and probably also to about 100 days. Before 40 days it is responsible for follicular development, and thereafter it is probably synergistic with eCG in causing marked ovarian activity.

6.2.3. Pregnancy Diagnosis

External signs

Absence of estrus is commonly used by owners as an initial screening method. However, some mares show estrous behaviour when pregnant and these mares may be mated, especially if restrained: this may cause embryonic death, if the cervix is opened during coitus – more likely in old or recently-foaled mares. It is commonly assumed that the mare will be in estrus 21 days after mating, and this is not necessarily true. On the other hand, non-pregnant mares may occasionally enter lactational anoestrus.

Clinical examination

At 18–21 days: good uterine tone and a tightly-closed cervix are indicators of pregnancy. At 21–60 days: good uterine tone, swelling at the base of uterine horns and tightly-closed cervix; all must be present for positive diagnosis.

By 60–120 days: swelling becomes less discrete, uterine horns become more difficult to palpate and uterine body becomes more fluid filled and prominent. The extension of the broad ligament between the uterine horn and the ovary (the mesosalpinx) is pulled into a tight band. Fetus can sometimes be balloted.

By 120 days to term: cervix becomes softer, fetus becomes more obvious. Dorsal surface of uterine body is always in reach. Fetus often felt moving after six months.

Ultrasound examination

From ten days after ovulation the conceptus can be imaged; it appears as a spherical anechoic structure approximately 2mm in diameter. The conceptus rapidly increases in diameter to reach approximately 10mm in diameter 14 days after ovulation. The outline remains circular presumably because of the thick embryonic capsule. Until day 16 the conceptus is mobile and may be identified either within the uterine horns or the uterine body. From day 17 until day 28 the increase in conceptus diameter is slowed. After fixation the conceptus rotates so that its thickest portion, the region of the embryonic pole, assumes a ventral position. From day 17 the conceptus appears triangular or flattened

in outline. The embryo may be imaged from approximately 21 days after ovulation when it appears as an oblong-shaped hyperechoic structure adjacent to the ventral pole of the conceptus. A heartbeat is commonly detected within the embryonic mass from approximately 22 days after ovulation. Growth of the allantois lifts the embryo from the ventral position and the allantois per se may be identified from day 24, when it appears as an anechoic structure ventral to the embryo. The size of the allantois increases and that of the yolk sac is gradually reduced until at approximately 30 days after ovulation they are similar in volume. From day 30 onwards, it is possible to image the amnion surrounding the developing embryo. At 35 days after ovulation the embryo is approximately 15mm in length and the allantois is three times the volume of the yolk sac. By days 38–40 the fetus is positioned adjacent to the dorsal pole of the conceptus. At day 40 the yolk sac is almost completely absent, and the umbilicus, which attaches to the dorsal pole, can be imaged. The umbilicus increases in length allowing the fetus to move to a ventral position within the conceptus. The fetus is positioned adjacent to the ventral pole from 50 days after ovulation. After day 50, limb buds can be readily imaged. The abdominal and thoracic portions of the fetus can be differentiated after day 50. Imaging of the fetal stomach is possible after day 60. The stomach is variably filled with anechoic fluid and can be detected caudal to the liver in more than 90% of fetuses. The fetal eyes may be imaged from day 60 and measurement of their diameter may be used to estimate the gestational age.

In a recent study on thoroughbred mares, out of 54 pregnancies, 28 (51.85%) pregnancies found in the right and 26 (48.15%) found in the left uterine horns. The embryonic vesicle was detected in all mares by the 15th day of pregnancy while the embryo was obvious by Day 21. Embryonic vesicle changed its shape from spherical on Day 15, oblong (36/54, 66.67%) or round (18/54, 33.33%) on Day 18 and changed to a guitar-pick or triangular shape by Day 21 of pregnancy. From Days 28 to 75 of pregnancy, the shape became irregularly spherical. The embryo and later the fetus changed its location form the ventral (Day 21), dorsal (Day 28 and 35), lateral (Day 45) to finally the ventral aspects of the gravid horn on Day 60 of gestation. Organization of the fetus into head, body, and limb buds was firstly observed in 64.81% of studied mares (35/54) by Day 45 and in all animals by the 60th day of pregnancy. Beginning of the ossification was observed in the head, ribs, and vertebrae in 87.03% of mares (47/54) by Day 60 of pregnancy. The fetal skeleton becomes visible during late pregnancy; the head, spinal column and ribs produce intense reflections that are easily identifiable. From 150 days onwards it is not always possible to image the entire fetus using high-frequency transducers because of their short depth of penetration. The dorsal portion of the fluid-filled uterus can always be imaged and the fetus may be seen by using a lower-frequency transducer either transrectally or trans-abdominally. From eight months of pregnancy it may be difficult to image more than a small portion of the fetus because of its large size. In the last trimester the amniotic cavity is increased in volume, and the amniotic fluid contains multiple, small, echogenic particles.

Endometrial glandular or lymphatic cysts are common in old mares. They are fluid filled and therefore appear anechoic when imaged with ultrasound. Cysts commonly have a fine, moderately-echogenic wall which may not be fully appreciated unless there are multiple cysts or there is free uterine fluid. Cysts may range from several millimeters to several centimeters in diameter. Luminal cysts may be confused with early conceptuses. To avoid these problems, it is prudent to record the size, shape and position of uterine cysts prior to breeding. Cysts do change in their appearance during

the year, however their position is constant. Additionally, cysts are often irregular in outline; cysts are frequently lobulated; cysts do not always have dorsal and ventral pole specular echoes; cysts do not change position; cysts do not increase in size; large cysts do not contain an embryo, whilst this can be seen in a conceptus after day 21.

Fetal sex may be accurately diagnosed 55–80 days after ovulation. Interpretation is difficult unless the operator is experienced. Diagnosis relies upon identifying the position and appearance of the genital tubercle. This is best done 55–75 days after ovulation. It is important to identify three structures: (1) Genital tubercle; (2) Umbilicus; (3) Tail. The genital tubercle is a bilobed structure approximately 2mm in diameter. In the male, the distance between the genital tubercle and the umbilicus is less than the distance between the genital tubercle and the tail. The opposite relationship exists in the female. Trans-abdominal ultrasound may be used for sex determination after nine months' gestation.

Laboratory methods

Progesterone concentrations in plasma (or milk) can be measured by R*adio-immunoassay* or *Enzyme-linked immunosorbent assay (ELISA) tests:* At 18–20 days post-ovulation pregnant mares should have plasma progesterone concentration above 1ng/ml *but* not all mares with high progesterone are pregnant (prolonged diestrus, early fetal death and mares with short cycles); mistiming of sampling (relative to previous ovulation) will give erroneous results; occasionally pregnant mares have low progesterone concentrations for short periods of time; thorough clinical examination gives cheaper and more complete and accurate information on the mare's reproductive status.

Equine chorionic gonadotrophin (eCG=PMSG) appears in the blood in detectable concentrations at approximately 40 days after ovulation and usually persists until 80–120 days after ovulation. The hormone is produced by the endometrial cups. The amount of eCG produced varies greatly from mare to mare, and mares carrying multiple conceptuses do not necessarily produce more than those with singleton pregnancies. eCG can be detected by radio-immunoassay, haemagglutination-inhibition test and latex agglutination test.

Placental estrogens reach peak concentrations in plasma and urine at 150 days, and concentrations remain high until after 300 days. Estrogens are tested for in the urine (free estrogens produce a colour reaction with sulphuric acid). The Cuboni test is the most accurate but involves an extraction procedure using benzene (carcinogen) and acid. The Lunaas test is simpler, uses acids but is sometimes difficult to interpret. *Plasma assays* for oestrone sulphate are commercially available.

6.3. Pregnancy in Cows

6.3.1. Development of the conceptus

The average length of gestation is regarded typically as 280–285 days. Gestation is often divided into three stages: (1) the ovum from 0–13 days, (2) the embryo from 14 days, when germ

layers begin to form until 45 days, and (3) the fetus from 46 days until parturition. The ovum begins to divide mitotically, a process known as cleavage, immediately after fertilization is complete. Division continues so that a solid cluster of cells or blastomeres known as a morula (mulberry shape) is formed by five or six days. The ovum enters the uterus 4–5 days after ovulation. From about day 6 after fertilization, the ovum begins to hollow out to become a blastocyst. This consists of a single spherical layer of cells, the trophoblast, with a hollow centre, but also with a group of cells, the inner cell mass at one edge. The inner cell mass is destined to form the embryo, whilst the trophoblast provides it with nutrients. At about day 8, the zona pellucida begins to fragment and the blastocyst 'hatches'. This is then followed by a period of blastocyst elongation. Development of the so-called germ layers begins from about the fourteenth day and characterizes the beginning of the embryo phase. The three germ layers arise from the inner cell mass and are termed the ectoderm, mesoderm and endoderm. The ectoderm gives rise to the external structures such as skin, hair, hooves and mammary glands and also the nervous system. The heart, muscles and bones are eventually formed from the mesoderm whereas the other internal organs are derived from the endoderm layer. By day 16, the embryo is sufficiently developed to signal its presence to the maternal system and prevent the luteolysis that would have occurred if the cow had not been pregnant. By day 45, formation of the primitive organs is complete and the fetal phase is considered to have begun. The embryo becomes attached to the endometrium by means of its membranes, through which nutrients and metabolites are transferred from mother to fetus and vice versa. The attachment process is known as implantation and may begin as early as day 20, although definitive placentation does not occur until day 40–45. The placenta is formed by intimate contact between the chorion and the endometrium. In ruminant species, the placenta is described as 'cotyledonary' since placental attachment occurs only in the discrete areas of the endometrial caruncles. Exchange of oxygen, carbon dioxide and nutrients between embryo and mother takes place solely through the cotyledons.

Fetal growth is increasing as pregnancy progresses. Pregnancy appears to occur more commonly in the right uterine horn than in the left at a ratio of 60 : 40, with the corpus luteum typically being on the same side, reflecting the slightly more active right ovary as reported by several authors. Approximately 1.4% of cattle births are twin births.

6.3.2. Endocrinology of pregnancy

Estrogen: Ovarian follicle development continues during pregnancy as a result of low levels of gonadotrophin secretion. Both ovarian follicles and the embryo-placental unit produce estrogens. Changing ratios of this estrogen and progesterone can cause some cows to show estrus during pregnancy, but this is not usually accompanied by ovulation.

Progesterone: Progesterone concentrations rise during the first few days of pregnancy in a similar manner to that occurring in the early luteal phase of the non-pregnant animal. The presence of the corpus luteum is necessary up to day 200 and the corpus luteum secretes progesterone throughout pregnancy. If cows are ovariectomized after 200 days, normal pregnancy will be maintained. There is evidence that in the pregnant cow major sources of progesterone during pregnancy are the adrenal glands and the placenta.

Gonadotrophins: Mean plasma LH concentrations are low during pregnancy. However, pulsatile secretion of LH does continue. This low rate of LH release must be sufficient to maintain progesterone production at least during early pregnancy, unless other luteotrophic substances are involved. There is little information on the release of FSH during pregnancy, but it is assume that it is secreted at relatively low levels. The placenta secretes a protein hormone, placental lactogen, which also has gonadotrophic properties.

6.3.3. Pregnancy Diagnosis

External signs

Non-return to service: A cow was diagnosed as non-pregnant if she was seen in estrus approximately 21 days after service. The percentage of cows not seen in estrus at about this time is known as the non-return rate.

Laboratory methods

Progesterone measurement: The measurement of progesterone to check for pregnancy offers the possibility of diagnosis at day 21 post-insemination. In the pregnant cow, blood and milk progesterone concentrations remain high between days 21 and 24 after ovulation, when they would be basal in the nonpregnant animal. Therefore a sample taken at this time may be used to discriminate between the pregnant and non-pregnant cow. The development of rapid highly sensitive ELISA procedures has allowed the commercial exploitation of this test. If the progesterone level is low, the cow is definitely not pregnant, provided that the right sample has been taken from the correct cow and the test has worked properly. If the progesterone level is high, the cow could be pregnant (80% accuracy). There are a number of reasons for false positive tests: (1) The cow may not be pregnant but the life of the corpus luteum may still be extended for some reason, such as a uterine infection; (2) The cow could have been inseminated at the incorrect stage of the cycle, i.e., during the luteal phase; (3) The diagnosis may be accurate, but the embryo or fetus is lost afterwards.

Pregnancy-specific proteins: Early pregnancy factor (EPF) is a pregnancy-dependent protein complex that has been detected in the serum of several species. It is detected using an immunological technique, the rosette inhibition test. It is claimed that this substance appears in serum shortly after conception and disappears very rapidly following embryonic death. Therefore the detection of EPF would offer a very valuable method of pregnancy diagnosis. Whilst a degree of success has been achieved using this technique in some laboratories, results in the cow have been insufficiently reproducible to offer much immediate hope of an improved method of pregnancy diagnosis. Two further proteins, bovine pregnancy-specific protein B (bPSPB) and bovine pregnancy associated glycoprotein (bPAG – an antigen synthesized in the superficial layers of the ruminant trophoblast), can be measured during early pregnancy in the cow with much more reliability.

Real-time ultrasound scanning

The availability of real-time B-mode ultrasound scanning has been a major advance in pregnancy diagnosis. Pregnancy can be detected as early as 17 days using this method. By day 19 the

amniotic sac has expanded considerably and therefore the lumen of the uterus can readily be observed. By day 22 it is possible to see the beating embryonic heart and by day 30 the conceptus is so obvious that a very rapid and accurate diagnosis can be made. It is the method of choice for early pregnancy diagnosis in the cow. For early pregnancy a 5 – 8 MHz linear transducer is required, whereas a 3.5 MHz transducer may be used for late pregnancies. Pregnancy can be detected as early as 17 days using this method. By day 19, the amniotic sac has expanded considerably and therefore the lumen of the uterus can readily be observed. Using Doppler, it is possible to identify the fetal heart from 6 – 7 weeks with a rectal probe. The advantage of using ultrasound scanning after insemination is that it enables relative earlier pregnancy diagnosis as well as offering high accuracy pregnancy diagnosis. Using real – time B mode, a 95% sensitivity on day 26 of gestation until an almost 100% sensitivity on day 29 is found. Ultrasound pregnancy diagnosis between day 10 and 16 has an accuracy of < 50 % and which improved by day 18 until day 22 (100%). Most of the embryonic deaths occur before day 25 but will continues thereafter, 10 – 15 % between 25 and 42 days, 6.3% between 42 and 56 days and 3.4% between 56 and 98 days. If early embryonic loss stays unnoticed, early pregnancy scanning could be a disadvantage and will reduce reproductive efficiency through extending the calving interval.

The embryo can first be seen by ultrasound scanning through the detection of the heartbeat, by some embryos heartbeats can be seen at day 19 but the heartbeats are generally visible at day 25. Although embryos can be seen by day 19 the most practical method is to scan pregnant animals expected to have embryos > 25 days. Fluid accumulation increases considerably at approximately day 25, thereafter the embryo detaches from the uterine wall and is easier to detect by ultrasound scanning. The uterine lumen contains a variable quantity of anechogenic fluid produced by the conceptus. After 27 days of pregnancy it is possible to confirm the pregnancy diagnosis by fluid accumulation in the uterine lumen. Fluid in the uterus during estrus may be confused with pregnancy and this may cause a potential diagnostic error. Before day 30 of gestation it is more difficult to observe the embryo because the young embryo is often located near the uterine wall in a small amount of fluid. Careful examination of the anechogenic area mostly reveals the presence of the embryo.

There are some landmarks for a veterinarian to detect normal development of the conceptus. After day 30 of gestation, it is possible to visualize the amniotic membrane. The amniotic vesicle is clearly visible through the reflections due to the round shape. After day 35, the first placentomes can be viewed. The attachment of the umbilical cord from the embryo to the uterus starts at day 40 of gestation. Ossified ribs in the fetus can be viewed from day 50 until day 60 of gestation. The sternum starts to ossify between day 81 and 85.

The first centers of ossification in the skull, which are very echogenic, appear at the end of the 2nd month of gestation. The ossification of the skull completes by day 100. The head of the fetus is visible by ultrasound scanning during the entire gestation. The eyes can be seen and are an indicator of the gestation length and increase rapidly during the first 6 months of gestation.

The ossification of the cervical, thoracic, lumbar and sacral vertebrae starts at day 61 to 65 whereas the ossification of the coccygeal vertebrae starts at day 86. The scapula, ilium and ischium ossified approximately at 70 days of gestation. The long bones of the limbs start to ossify around day

61 – 65 and the digits begin to ossify between day 81 and 85 of gestation. A relationship between multiple fetal structures and the gestation age which can be used to estimate the gestation age was found.

The heartbeat is easy to visualize with the ultrasound. The frequency of the heartbeat varies with the gestation age. Moreover, within one fetus the heartbeat varies at one day due to individual fetal activity.

During the first stage of gestation the abdomen is almost entirely filled with the liver and the mesonephros, during the last stage the liver growth slows down. Around 60 days the stomach is divided into the four compartments whereas the abomasum and the omasum appear as hyperechogenic spots and the rumen as a hypoechogenic spot. Other organs in the abdomen are not that easy to identity. In the first part of gestation the kidneys occupy a large volume in the abdomen, around day 90 of gestation the kidneys get their 'normal' location and shape.

Examination of the cow for ultrasound fetal sexing can be done between 54 and 100 days of pregnancy, but the ideal moment is between 60 and 70 days of gestation..

Rectal palpation of the fetus

In the nonpregnant and early pregnant animal the uterine horns can be felt to be approximately equal in size and diameter. It is possible to detect a difference in the size of the two horns from about day 40 of pregnancy onwards. This is easier in heifers than in multiparous cows and is to a large extent dependent on the skill of the operator. If, from about day 30, a fold of the uterine wall is picked up and then released, the sensation of two layers of tissue passing through the fingers may be felt. This so-called fetal membrane-slip (FMS) is due to the presence of the chorioallantois inside the horn. From 6–8 weeks the disparity in horn size becomes quite marked, the pregnant horn becoming up to six times the diameter of the non-gravid horn at 10 weeks. After this stage, the non-gravid horn also begins to enlarge due to the invasion and attachment of the placenta throughout the uterine lumen. By 12 weeks, the diameter of the pregnant horn may be 10 cm or more and is easily detectable. As the gravid uterus becomes larger and heavier it begins to sink down below the pelvic brim and by the fifth month it may not be possible to palpate it. Only when the fetus has grown further, to the seven-month stage, may it be possible to feel it again. However, in these cases pregnancy may be suspected by the absence of the normal reproductive organs in the pelvic cavity and the palpation of the cervix and anterior vagina as a taut band of tissue passing over the pelvic brim. After the seventh month of gestation, the fetal head can usually be palpated with ease. Another indication of pregnancy may develop about mid-term. The placentome could be palpated as circumscribed structures (about 1 cm by day 90 of pregnancy) if the operator pick up a part of uterus. The size increases progressively by advancing of pregnancy. Blood flow through the middle uterine artery on the pregnant side increases dramatically during pregnancy. This usually pulsates like a normal artery, but in advanced pregnancy (from 120 d onwards) slight compression of this reveals a characteristic vibrating sensation known as fremitus. Certain pathological conditions of the uterus can be confused with a normal pregnancy by causing uterine enlargement. These include pyometra, large tumours and the presence of a mummified fetus.

Estrone sulphate

The identification of compounds produced by the conceptus rather than by the dam would have clear advantages in the diagnosis of pregnancy in farm animals. Estrogens are produced by the bovine conceptus and oestrone sulphate concentrations in maternal plasma rise from about day 70 of pregnancy. The oestrone sulphate content of milk reflects that of plasma and it has been found that a positive oestrone sulphate test at 15 weeks of pregnancy gives a 100% accurate diagnosis of pregnancy. In most cases, detection of non-pregnancy at this late stage is of limited value to reproductive management, but it can be used as a confirmatory test following an earlier positive diagnosis, such as a milk progesterone test at 21–24 days. If there is any reason to suspect that a cow has aborted, the oestrone sulphate test can be very useful in determining her status.

Mammary development

Mammogenesis or development of the mammary gland occurs as a consequence of pregnancy and in the primiparous heifer changes can be detected as early as four months of gestation. In addition, a viscous brown secretion may be expressed from the teats. Of course, these changes are not apparent in the parous, lactating cow. Steroidal-type growth promoters can elicit identical changes in the mammary glands of heifers. This can be an additional confounding factor in countries and situations where they are used. It is only during the last few days of pregnancy when the udder becomes distended with colostrum that mammary development can be regarded as an accurate diagnosis of pregnancy.

Ballottement

The abdomen of the pregnant animal begins to become distended from about seven months of gestation. If a hand is pushed firmly against the right side of the abdomen, the fetus may sometimes be felt to rebound against it. This technique is commonly used in cattle markets by prospective purchasers. However, it is not a reliable indicator of pregnancy particularly if the findings are negative.

6.4. Pregnancy in Buffalo-Cows

6.4.1. Development of the conceptus

The gestation period ranged from 312 to 321 days with an average of 316 days. The birth weight of young ranged from 33 to 40 kg. Embryonic development in buffaloes is advanced by 12–24 h compared with embryonic development in cattle and this has been demonstrated by both *in vivo* and *in vitro* studies. The advanced embryonic development in buffaloes is associated with earlier entry of buffalo embryos into the uterus on around days 4–5 after estrus and while in cattle, embryos are located in the uterus at around day 6 after estrus. Compact morulae in buffaloes are observed from day 5 after estrus and blastocysts typically from around day 6. Hatching of *in vivo* derived buffalo embryos was reported to occur from day 5 to day 7 in different studies. The apparent variability in the

time of hatching of blastocyst embryos could be related, at least in part, to the relatively diverse genotypes (riverine, swamp and crossbred buffaloes) studied. Embryo elongation in buffaloes is thought to occur from around day 13, the pre-attachment phase from day 17 to day 24, and the transitory attachment phase is initiated around day 25. Pregnancy-associated glycoprotein β is also reported to increase from day 25 in buffaloes.

Slaughterhouse studies of fetuses from Egyptian and Indian buffaloes indicated that the body as a whole is increased steadily in size. However, the rate of increase varied from one measurement to the other. The measurements of the head showed widely variable rates of growth. Both the chest and abdomen circumferences showed lower rates of growth. Organization of embryos into head region, forelimb-buds, and tail spouts occurred between Day 38 and 50. The caruncles in the gravid uteri of the buffalo cows are continued in growth almost until parturition. Ossification of the skull, ribs, and vertebrae were demonstrated between weeks 8 and 10 of pregnancy. Both the allantoic and the amniotic fluids were clear and watery in viscosity during early pregnancy. The viscosity of the amniotic fluid changed to thicker state at the eighth month and became syrupy during the ninth and tenth months. Increasing the turbidity of the amniotic fluid may be attributed to an increase in the secretion of the respiratory tract and buccal cavity, which possibly accounts for the increased viscosity and improved lubricated properties of the fluid. The fetal anterior and posterior presentation occurred in equal frequencies. During the time from the fifth and sixth months, the body length of the fetal calf exceeds the width of the amnion and thus, at this stage the final polarity of 95% of fetuses in anterior presentation is adopted

6.4.2. Endocrinology of pregnancy

In pregnant buffalo, peripheral plasma T4 levels fluctuated slightly throughout pregnancy without exhibiting a specific trend. The magnitude of T4 levels was significantly lower in buffalo than in the cows throughout pregnancy and that the hormonal patterns of the two species were significantly different during gestation. It was hypothesized that T4 requirements for the fetal buffalo calf may be lower than that for the fetal cattle calf since the buffalo gestation period is a month longer and the metabolic rate lower than in the cow.

In a previous study, an exponential increase in estrone sulphate levels was also recorded in buffaloes beginning at the fourth month of gestation. However, the mean hormone levels in this species, after initially being lower than Sahiwal and Karan Swiss cows up to 6 months of pregnancy, increased to higher levels thereafter. From this study it was concluded that in addition to genetic factors, environmental adaptation could also influence the oestrone sulphate levels. In addition, the study also provided a basis for pregnancy confirmation by milk oestrone sulphate determination after 110 days in gestation in cattle and buffaloes using a simple, direct, assay procedure.

The concentration of plasma progesterone in buffalo cows declined from 3·0 ± 0·2 ng/ml (mean ± S.E.) during days 21–17 before calving to 2·1 ± 0·1 ng/ml at day −3, followed by a rapid fall during the last 3 days of gestation, reaching 1·2 ± 0·3 ng/ml at the day of parturition. During the postpartum period, plasma progesterone decreased gradually and reached baseline values after day 15 postpartum, when the residual corpus luteum of pregnancy had completely regressed in all the animals

studied. The plasma concentrations of total estrogens started to increase at day −15 in cows and day −5 in heifers from below 40 pg/ml to 122·5 ± 51 pg/ml for cows and 100·7 ± 3·4 pg/ml for heifers by day −1. This was followed by a sharp increase to 251·2 ± 17·3 and 240·5 ± 10·1 pg/ml in these animals at the day of calving. Immediately after parturition, total oestrogens dropped abruptly to the lowest values and remained below 30 pg/ml in all cows and heifers until the end of the sampling period.

6.4.3. Pregnancy diagnosis

Uterine changes during pregnancy

The biometry of the buffalo uterus was studied in 54 and 167 non-gravid and gravid buffalo uteri, respectively. The material covers the period from the 38th day until some two weeks before parturition. The non-gravid uterus averaged 525 gm. in weight. The two horns are nearly equal in weight and size. The total number of caruncles averaged 100 and the counts were equally shared by both horns. In the gravid uteri, right side pregnancy was observed in 53% of the total pregnancies. The weight of the uterus and its contents increases 63-fold during the period studied and is 88 times that of the non-gravid state. This increment is due to the increase in the different components and the percentages were variable from one month to the other. The empty uterus formed about 74% of the weight of the uterus and its contents at the second month and had fallen to 15% at the tenth month. The empty uterus increased about 13 times from the second to the tenth month. Both the gravid and the non-gravid horns increased progressively in weight during the different months of gestation. The text contains the details of the data concerning the different measurements of the gravid and the non-gravid horns. The greater curvature of the gravid horn increased 63-fold from the non-gravid state and 50 times from the second to the tenth month. The circumference increased 82 and 62-fold during the given stages, respectively. The weight of a single caruncle increased some 48-fold from the third to the tenth month.

Rectal palpation

The sensitivity of transrectal palpation for detecting pregnant buffalo-cows was 37.5% at days 31-35, increased to 93.8% at days 46-50 and reached 100% at days 51-55. The specificity of transrectal for detecting non-pregnant buffalo cows ranged between 90.9%, and 100% between days 31 and 55. It have been concluded that transrectal is an accurate method for diagnosing pregnant and non-pregnant buffalo cows from day 46 after breeding.

Ultrasound

In a detailed study of the fetal dynamics in buffalo cows, the embryo and amniotic vesicle (AV) were detected in all buffalo-cows by the 4^{th} and 5^{th} week of pregnancy, respectively. Organization of the embryo into head, body and limb buds was firstly observed in 33% of animals by the 6^{th} week and in all animals by the 7^{th} week. Beginning of the ossification was observed in the head, ribs and vertebrae in most animals by the 8^{th} week and in all animals by the 10^{th} week. The placentomes were firstly observed by the 10^{th} week as small hyperechogenic nodules. The omasum was first detected as circumscribed hyperechogenic structure by the 10^{th} week. The amniotic fluid remained clear up to the 14^{th} week of pregnancy, before it became slightly turbid, while definite

turbidity was observed by the 30th week. The allantoic fluid remained clear throughout the whole observation period. The fetal parts and organs developed differently, with increasing (crown-rump length, CRL; biparietal diameter, BPD; abdominal diameter, ABD; omasal diameter, OMD; and thorax height diameter, THD) or decreasing (amniotic vesicle, AVD; uterine diameter, UTD; ruminal length, RUL; eye-ball diameter, EBD and placentome diameter, PLD) growth rate with the progress of gestation. Gestational age affected the possibility of the ultrasonic fetal sex determination Fetal sexing was unreliable before the 8th week of gestation. The possibility increased through the 10th week, remained constant between the 10th and 14th weeks, and decreased gradually until the 24th week of pregnancy. Afterwards, fetal sexing was impossible due to the far position of the fetus. The fetal gender was correctly assigned in 68 of the 70 possibly diagnosed occasions (overall accuracy 97.1%), where the transrectal ultrasound examination findings were confirmed by the direct observation of calves at birth. In male fetuses, the genital tubercle (phallus) was a hyperechogenic, oval or sometimes round structure, located cranial to the hind limbs and at the abdominal attachment of the umbilical cord. In female fetuses, the genital tubercle (clitoris) was located behind the hind limbs and toward the base of tail. The fetal scrotum could be observed between the two hind limbs first by the 10th week of gestation. The fetal udder/teats were first viewed between the hind limbs also by the 10th week. The testes were not observed in the scrotum during the observation period. Early in gestation (8th week), fetal sexing depended only on the view of the fetus in the frontal plane. The importance of this view is decreased with pregnancy progress. Contrariwise, the importance of the sagittal view is increased with gestation progress. The cross-sectional plane could be used from the 10th to 18th weeks of gestation. The buffalo-fetuses changed their presentation frequently until the 28th week of pregnancy. From the 30th week onwards, all the fetuses were turned into the anterior presentation.

6.5. Pregnancy in Ewes and Does

6.5.1. Development of the conceptus

Certain gross external features were studied in 144 caprine and 109 ovine foetuses to aid in their age estimation. Body size changes were compared to ascertain phylogenic allometric relationships between the two species. Developmental patterns of the integument, external jugular, facial and scrotal veins and male and female external genitalia followed the same time-event and sequence in both species. Onset and sequential changes of bone formation in the calvarium (skull-roof), and sequence and completion of regional hair distribution as well as the time-event of teeth eruption were similar in both species. However, the time limit for the whole calvarium to become hard and the onset of regional distribution of hairs varied. Body weight (BW) and crown-rump length measurements showed that a caprine fetus weighing 51.13 g and measuring 11.27 cm at 6–8 weeks attained a weight of 1371 g and length of 31.69 by 18–20 weeks. An ovine fetus, however, was of larger body size (6–8 weeks, 66.8 g and 12.64 cm; 18–20 weeks, 2111 g and 43.67 cm). However, relative growth changes in these parameters indicated that both species maintained similar and proportionate linear growth relationships from 6–8 to 18–20 weeks of gestation (caprine, $y = 57.4x–728.1$, $r = 0.924$; ovine, $y = 64.8x–941.8$, $r = 0.965$; where x is crown-rump length and y is body weight). It was concluded that, to estimate fetal age, external features which form essential

components of the body are more reliable criteria than those which are guided largely by environmental and nutritional factors. Also, in general, sheep and goats seem to maintain a very close phylogenic relationship in both developmental characteristics and body shape and form.

One-hundred and ten pregnant Targhee ewes that had been mated to Suffolk rams were killed at different stages of gestation. Mammary glands were removed and the gravid uterus was dissected into fetus, membranes, fluids and uterus components. The water, fat, ash, nitrogen and energy contents of the different components were determined. The uteri and mammary glands of 28 non-pregnant ewes were also analyzed for chemical composition. Relationships were derived to describe growth and retention of the different nutrients in the various products of conception during pregnancy. Single and twin fetuses averaged 0.15 and 0.30 kg (total weight) at day 70 of gestation; and 6.21 and 10.64 kg at day 140, respectively. Growth rates of single fetuses averaged 31, 71, 129 and 199 g/d while those of twin fetuses averaged 47, 153, 236 and 202 g/d at days 80, 100, 120 and 140, respectively. Fetus(es) as a proportion of total conceptus weight increased from 14% at day 70 to 70% at day 140, while membranes and fluids decreased from 37 to 8% and 49 to 22%, respectively, over the same period. There were no significant differences due to number of fetuses carried. From day 70 to day 140 of pregnancy, fat content of the fetuses increased from 0.5 to 2.1%; fat-free organic matter increased from 7.3 to 13.2%; water decreased from 90.4 to 81.7%; ash increased from 1.9 to 3.0%; nitrogen increased from 1.0 to 2.0%; and energy increased from 0.45 to 0.93 kcal/g.

6.5.2. Endocrinology of pregnancy

In goats, a gradual increase was observed in immunoreactive (ir-) inhibin, with maximal levels at the 17th week. The plasma concentrations of estradiol and prolactin (PRL) showed nearly similar patterns during pregnancy, where they declined to basal levels during the first 4 weeks post-breeding and then increased significantly, with the maximal concentration during late pregnancy. The plasma FSH and LH concentrations were maintained at basal levels throughout the gestation period. The plasma progesterone concentration abruptly increased in the first week post-breeding and remained at high values throughout the pregnancy period. Immunohistochemical localization of inhibin alpha, β_A, β_B and steroidogenic enzymes cytochrome P450 aromatase, 3alpha-hydroxysteroid dehydrogenase (3betaHSD), cytochrome 17alpha-hydroxylase P450 and cholesterol side-chain cleavage cytochrome P450 in the cyclic and pregnant goat CL revealed positive immunoreactivity without affinity differences between the luteal and pregnancy stages. The placental syncytiotrophoblasts also showed positive staining, except for inhibin $beta_A$ and 3betaHSD. The giant binucleate cells of the placenta showed positive immunoreactions to PRL. These results suggest that the high concentrations of ir-inhibin, estradiol and PRL during late pregnancy are of placental origin and that the placenta may have a vital role in the maintenance of pregnancy, regulation of mammary growth and preparation for kidding and lactation in goats.

6.5.3. Pregnancy diagnosis

Pregnancy scanning is a great management tool to improve profits for meat and wool enterprises, particularly those running higher stocking rates and when paddock feed is in short supply.

Ultrasound

A study used 336 ultrasonographic examinations obtained from 12 ewes between D 22 and 146 of pregnancy was performed. The mean duration of pregnancy was 151.2 ± 2.3 days (range 148 – 154 d). Accessibility of the different fetal organs and parts for ultrasonic examinations depended on day of gestation. The CRL and AVD were accessible only during the first trimester of gestation. Thereafter, they became too long to be presented efficiently on the screen as a whole. The CHD, ABD, and RUL could be scanned during the second trimester. The PLD, EBD, and KIL were within the range of the ultrasound beam during the second and third trimester. Accessibility during the total gestational period was 17.3% (58/336) for the CRL, 16.9% (57/336) for the AVD, 19.6% (66/336) for the CHD, 16.7% (56/336) for the ABD, 27.7% (93/336) for the RUL, 52.7% (177/336) for the PLD, 37.8 (127/336) for the EBD, and 21.4% (72/336) for the KIL. Also, accessibility of the various fetal parts for scanning depended on the method of examination. Transrectal scanning could be performed efficiently until D 48 of pregnancy. Beyond this time most of the fetal parts, except the placentomes and fetal head, became out the range of the ultrasound beam. Trans-abdominal scanning could be done as early as 24 days after breeding, however, by this time the scanning view was much inferior to that of the transrectal one. By advancing of pregnancy, the view became more better and equivalent to the transrectal one.

The embryo was first detected on D 25.38 ± 1.2 (range 24 – 27 days). The AV was firstly detected on D 28. Differentiation of the embryo into head, body, limbs and tail was noted on D 38 ± 3.2 (range 35 – 41). Ossification was first observed in the fetal head, ribs and vertebrae on D 44.86 ± 2.5 (range 42 – 48), 50.25 ± 3.4 (range 46 – 55), and 51.50 ± 2.5 (range 48 – 55), respectively. The amniotic fluid contained no reflecting particles and gave almost black image until D 80.67 ± 11.2., when many echogenic spots were observed whirling around on it. Definite turbidity was observed by D 103.0 ± 8.0. The allantoic fluid remained clear without any echogenic particles until D 138.0 ± 9.5, when few echogenic spots appeared moving within it. Increasing the turbidity of the fetal fluid late in pregnancy might be due to an increase in the secretion of the respiratory tract and buccal cavity, which possibly accounts for the increased viscosity and improved lubricated properties of the fluid. Time of organization, ossification and change the echogenicity of the fetal fluids might help in judging the fetal age.

Except the placentome diameter, all regression and correlation coefficients are highly significant. Fetal age could be estimated from visual inspection of these figures, but for accurate estimation, references should make to the equations, which have been made computed with the size measurements as the independent variate.

The placentome was the most available structure of the pregnant uterus for ultrasound examination. It was first observed on D 37.29 ± 5.1 (range 30 - 42) as small slightly elevated echogenic areas on the surface of the endometrium. Previous reports noticed it between D 30 and 40. This time, its echogenicity resemble to a large extend that of the uterus. By advancement of pregnancy, it became more echogenic and brighter and appeared as cup or C-shaped in cross section. This is similar to earlier anatomical and ultrasonographic studies. During the last month of pregnancy,

it became less echogenic, while its attached surface appeared more echo-intensive. By this time, it started to collapse with reducing its lumen, while in areas adjacent to the fetus it appeared flattened. Collapsing of the placentome might be an indication for occurrence of a degenerative process. On the other hand, these changes in the placentomes may be associated with increased placental perfusion and tissue permeability during late pregnancy.

The KIL could be used as a biometric index predicting the GA in sheep. The fetal kidney could be visualized first by D 73.2 ± 6.3 (range 62 – 76). The echo-poor fluid filled rumen served as a landmark for detection of the left kidney. Early in its detection, the kidney was ill distinct, had no sharp demarcation and its echogenicity resemble to large extend that of the liver. Later on (from D 90 onwards), differentiation may be made between the renal medulla containing the more transonic discrete pyramids separated by septa (columns of Bertin) and the cortex consisting of a uniform distribution of small discrete low-level echoes. Its mean long axis increased from 1.41 ± 0.2 cm on D 76 to 3.61 ± 0.3 cm on D 146. Using the KIL – predicting equation ($Y = 0.0345X-1.2052$, $R^2 = 0.81$), a difference of ± 7 d between the expected and observed fetal age was found in 16 sheep (53.3%) and ± 14 d in another 14 sheep (46.7%). The difference between the expected and observed fetal ages was not significant. In Human, KIL was measured by high resolution transvaginal ultrasonography between 14 and 17 weeks' gestation, and by transabdominal ultrasonography beyond 18 weeks' gestation. One or both kidneys could be seen in 95% of cases after 20 weeks of gestation.

A total of 110 does aged between 8 and 36 months were used to study fetal development. The accuracy for detecting early pregnancy (fetal fluids and heartbeats) and fetal number (single or twins) was measured. The relationship between gestation age and CRL or BPD was determined from day 40 to 109 of gestation. The accuracy of fetal sexing was determined by differentiation of genital tubercle (GT) at different stages of gestation (from day 40 to 109 of gestation). The examination revealed 95.5% of examined does were pregnant, with accuracy 100% in detecting pregnancy for positive cases. The fetal number was 45.7% and 54.3% for single and twins or more, respectively. The trans-rectal probe (TR) enabled the reliable and earlier recognition of fetal fluid and heartbeats (indicating pregnancy) than Trans-abdominal (TA) probe. For maximum reliability, the TR observation of heartbeats is recommended as conclusive evidence of the presence of a live fetus. The TA convex probe was used from day 40 to 89 for measuring CRL and from day 40 to 109 for measuring the BPD. Gestation equations were: $CRL = 0.464x-17.767$ and $BPD = 0.055x-1.431$ (x = gestational age in days). The relation between gestational age and CRL or BPD was highly significant. The fetal sexing was found in 100, 83.3 and 64.3% of single pregnancies and in 85.7, 80 and 52.3% of twins or pregnancies during 40–60 days, 61–70 days and 71–109 days of gestation, respectively. The accuracy of sex identification among the 3 groups was not significantly higher in single than twins or more pregnancies. However, identification of GT in male fetus was possible from day 40 onward. From a total of 105 scanned does, 80 (76.25) were sexed. In conclusion, B-mode real-time ultrasonography is recommended as a reliable mean for early detection of gestation as early as 19–27 days after mating, for CRL or BPD measuring as well as fetal sex determination from day 40 of gestation onwards under field conditions.

Suggested Readings

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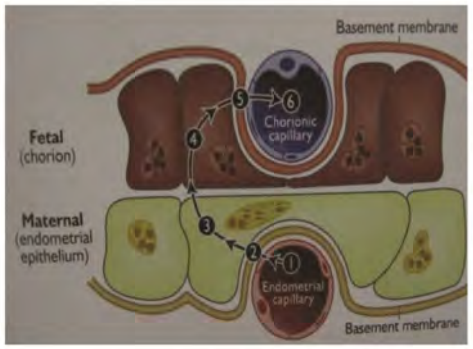
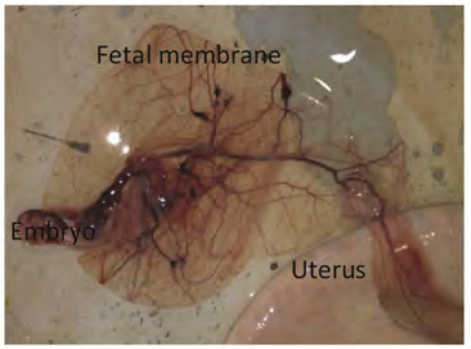
APPENDIX:Tables and Figures

Table 12: Pregnancy characteristics in farm animals

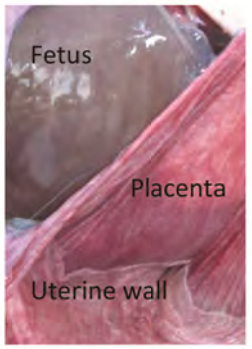
	Female camels	mares	cows	Buffalo-cows	Ewes and does
Ovum in the uterus after ovulation	6-7 days	5-6 days	4-5 days	4-5 days	5-6 days
Type of placenta	diffuse epitheliochorial	diffuse epitheliochorial	Cotyledonary epitheliochorial	Cotyledonary epitheliochorial	Cotyledonary epitheliochorial
Maintain of pregnancy	ovarian progesterone	Placental progesterone	ovarian and placental progesterone	ovarian and placental progesterone	Ewe: placental progesterone; Doe: ovarian progesterone
Side of pregnancy	Left horn 99%	Left horn 60% Right horn 40%	Left horn 40% Right horn 60%	Left horn 40% Right horn 60%	Multiple pregnancy (right + left horns)
Pregnancy diagnosis	Corpus luteum 14d Uterine changes 45d Ultrasound: 14-21 d	Uterine changes 30d Ultrasound: 14d	Corpus luteum 21d Uterine changes 33 d Ultrasound: 24-28 d	Corpus luteum 21d Uterine changes 40-45 d Ultrasound: 28-35 d	Ultrasound: 20-24 d



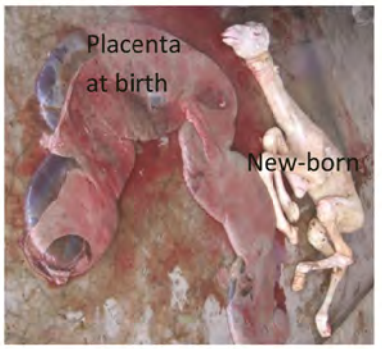
A) Epithelio-chorial placenta: 6 layers separte between fetal and maternal bloods



B) Camel embryo (35d) with the fetal membranes

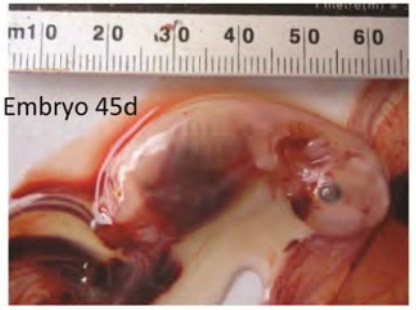


C) Diffuse placenta of camel: the way of contact



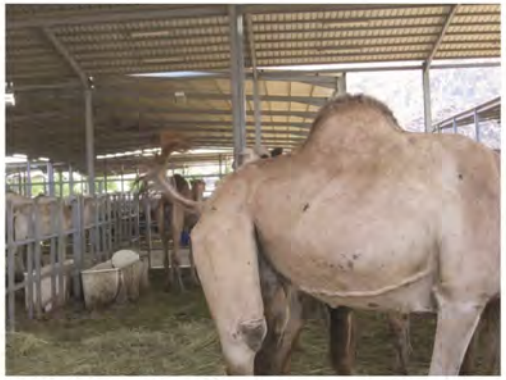
D) placneta of camel at birth

E) The third fetal membrane (epidermal membrane which present only in camel



F) Debelopment of the camel conceptus

Fig. 33: Placentation and fetal development in dromedary camels (*Senger, 2003; Makka – Qassim, KSA, 2010 - 2012*).

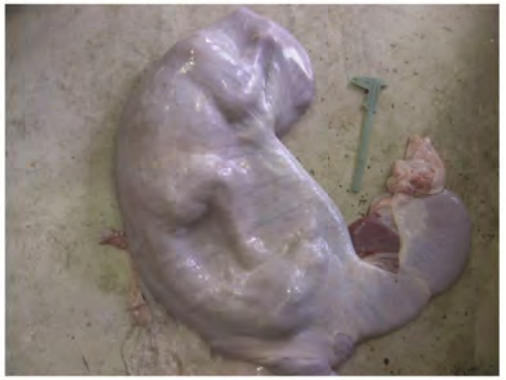


A) Raising the tail when approached by a male or persons – startng 2w after mating

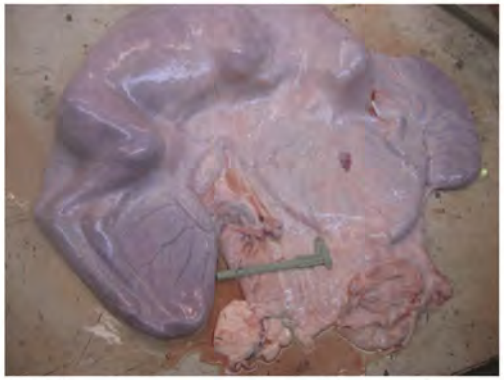
B) Enlagment of the abdomen – 12m

C) Odema of the udder – 10m

D) Asymmetry between left and right horns- 3m



E) Progessive enlargment of the left horn – 7m



F) The uterus near parturition – 12.5 m

Fig. 34: Changes during pregnancy in female camels; external signs (A,B,C) and internal changes (D,E,F) (*Makka – Qassim-KSA, 2010 – 2013*).

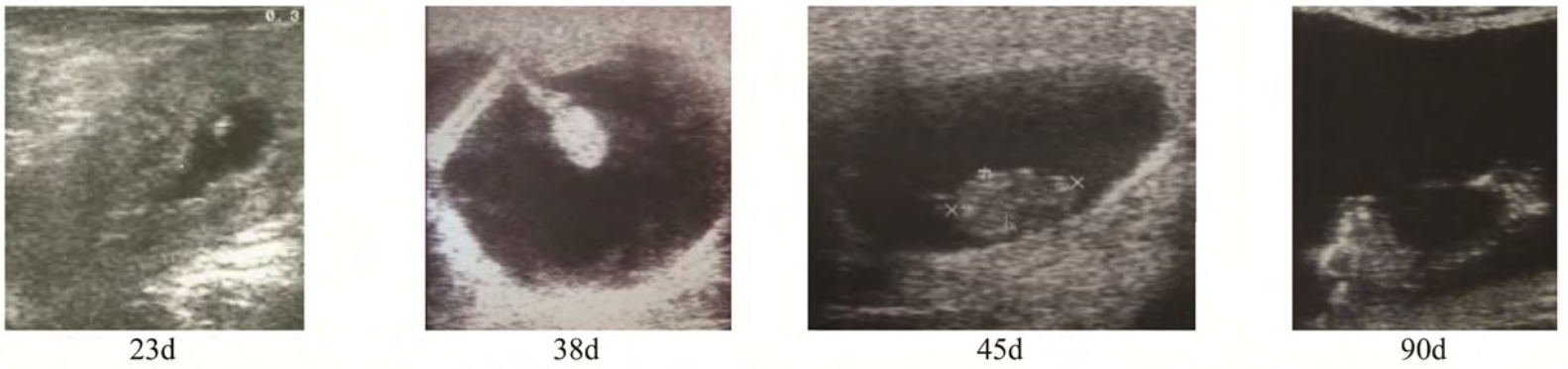


Fig. 35: Ultrasonographic early pregnancy diagnosis in female camels (Qassim-KSA, 2007-2013).

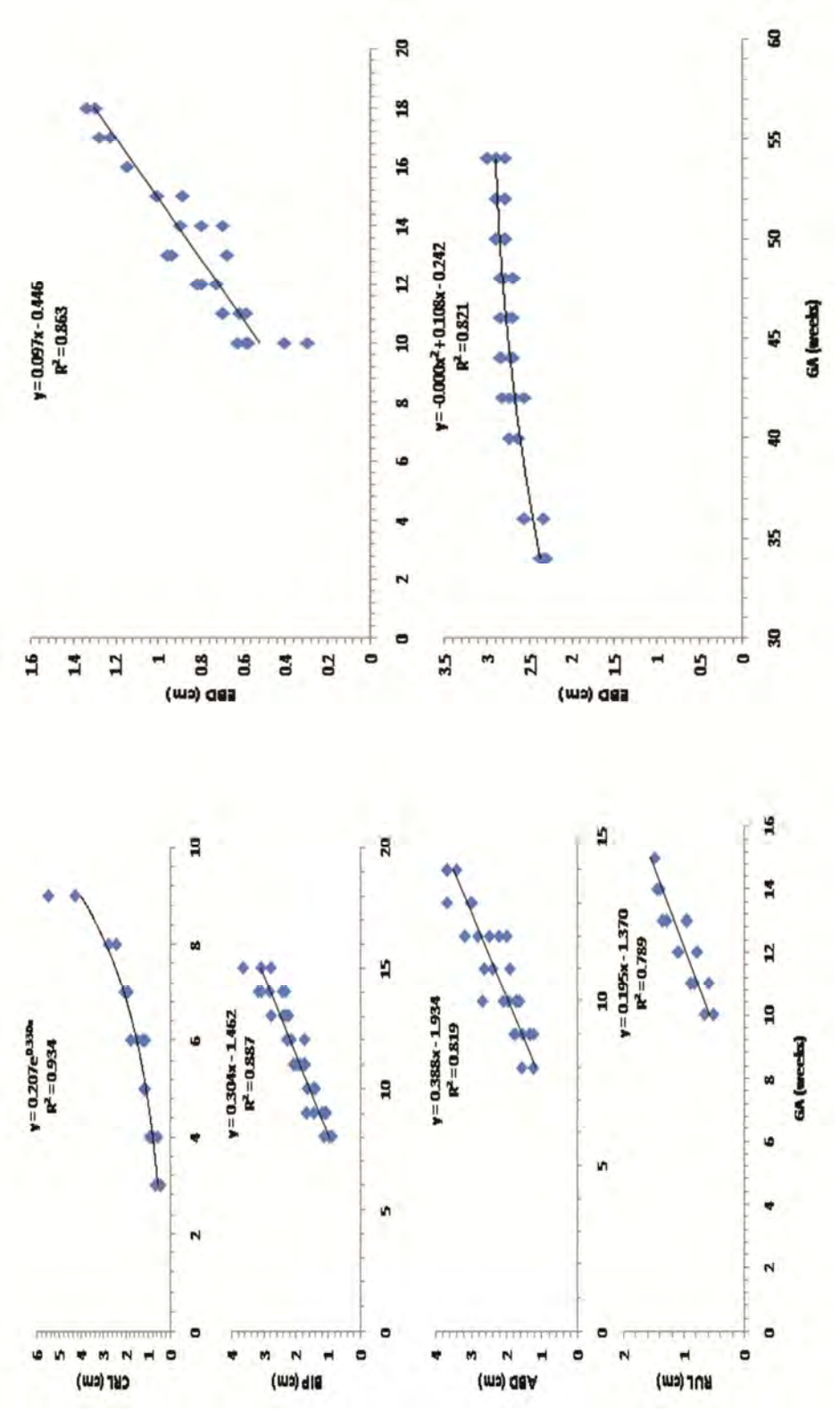
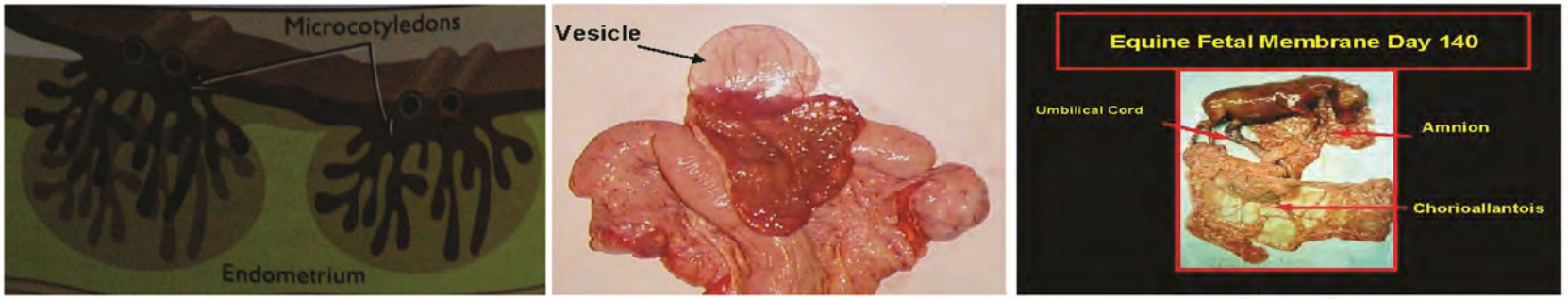


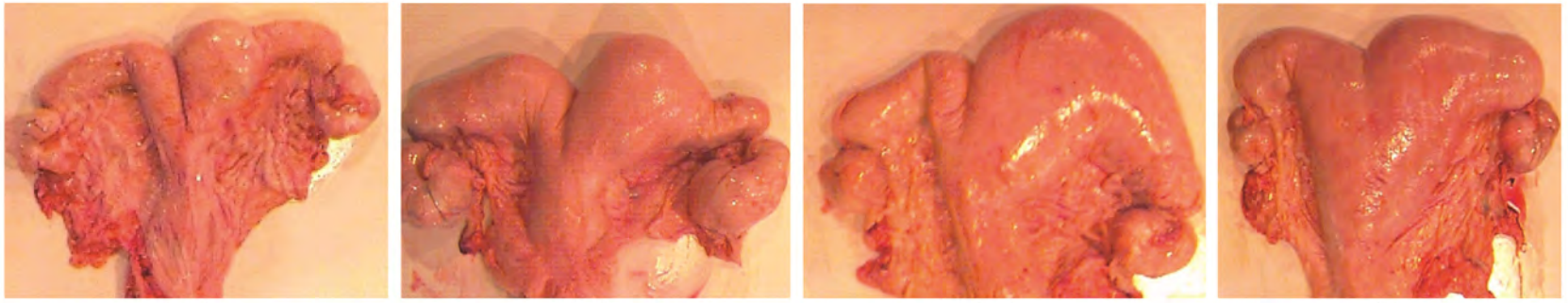
Fig. 36: Pattern of development of different parts of camel fetus during different gestational period (GA) and equations for predication of GA (*Qassim-KSA, 2013*).



A) Epithelio-chorial pkacents B) Amniotic vesicle: 25d C) The fetal membranes

Fig. 37: Placentation and fetal development in mares (*Senger, 2003;*

***http://www.vivo.colostate.edu/hbooks/pathphys/reprod/placenta/equine.html*).**



32d 45d 60d 80d

Fig. 38: Changes during pregnancy in mares (*Senger, 2003*).

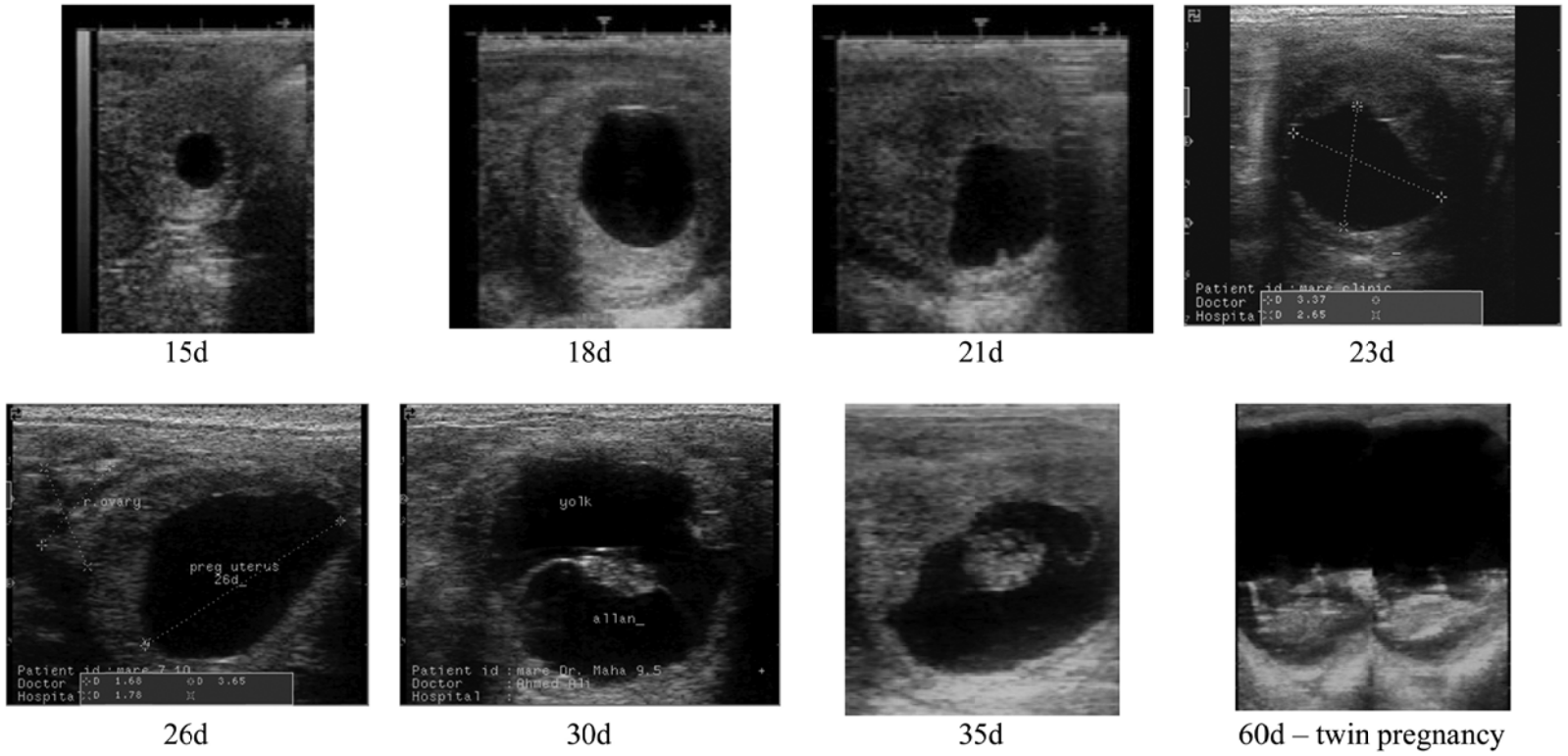


Fig. 39: Ultrasonographic early pregnancy diagnosis in mares (*Assiut-Egypt, Japan, 2000-2007*).

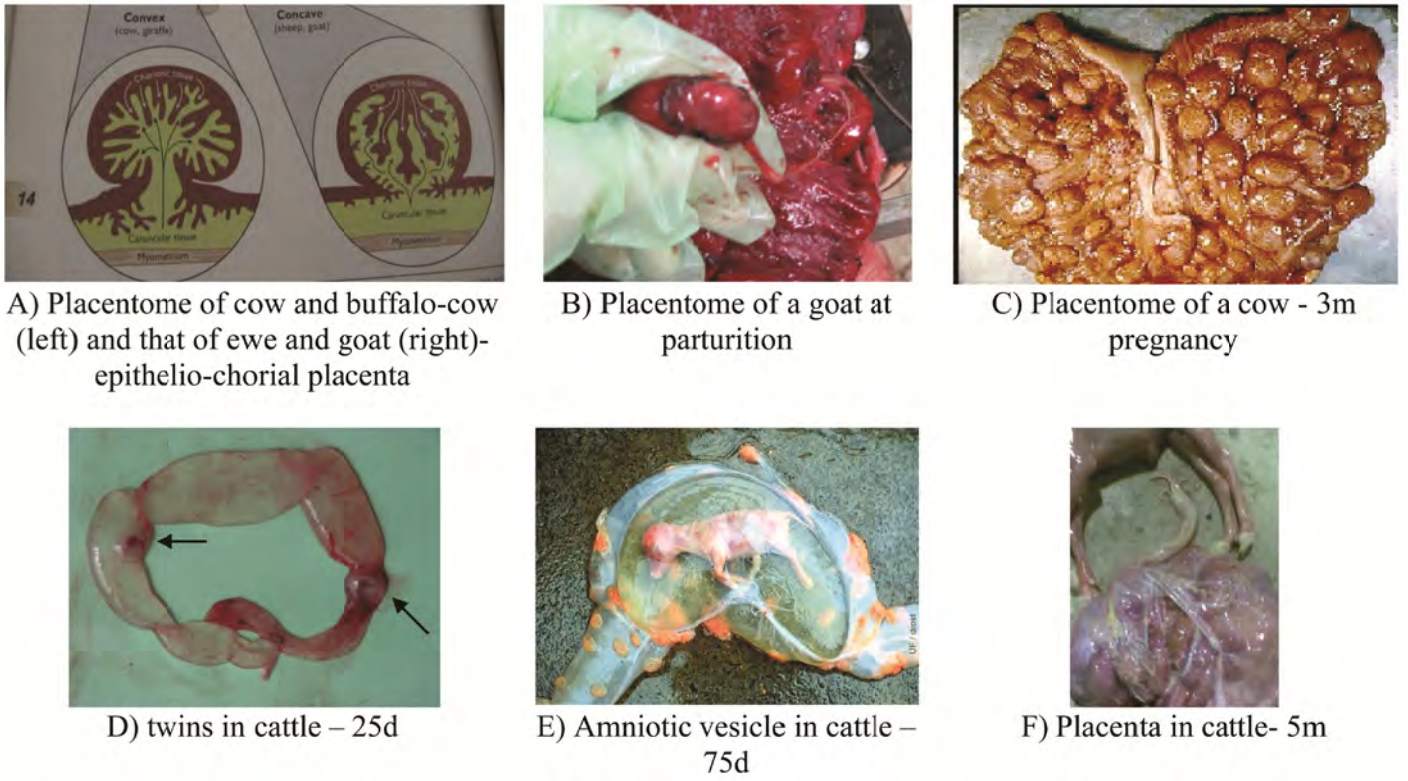
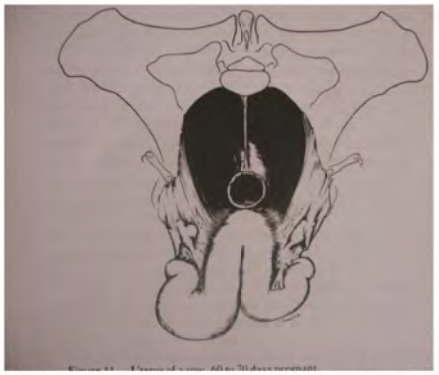
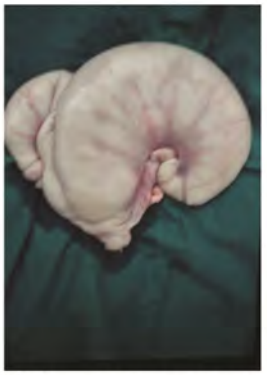


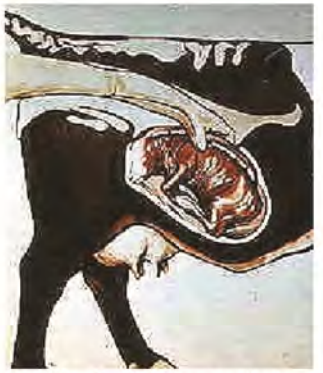
Fig. 40: Placentation and fetal development in cows, buffalo-cows, ewes, and does (*Senger, 2003; Qassim-KSA, 2009; Makka, 2010*).



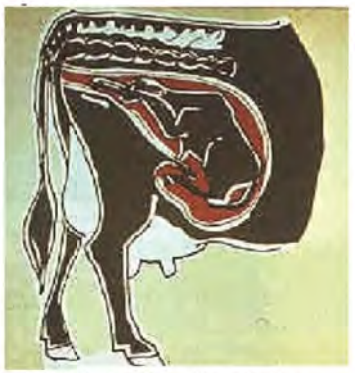
60d: the uterus in the pelvic cavity with clear asymmetry



90d: increase asymmetry between right and left horns

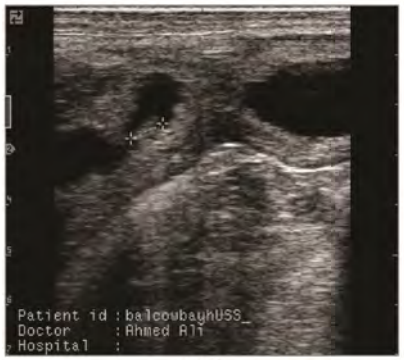


150d: the fetus touch the abdominal cavity – sinking stage

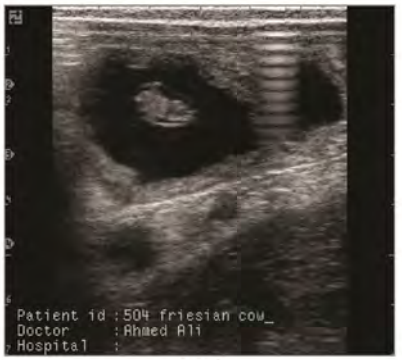


240d: the head and fore-limbs enter the pelvic cavity

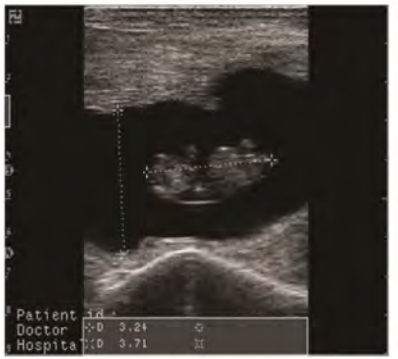
Fig. 41: Changes during pregnancy in cows (*Roberts, 1986; Assiut-Egypt, 1990*).



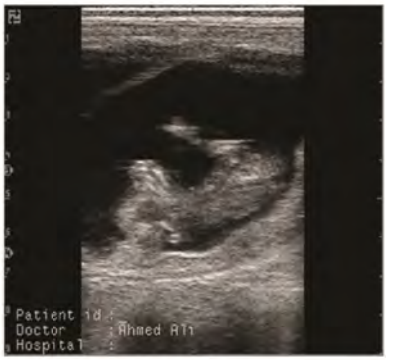
28d



38d



45d



55d

Fig. 42: Ultrasonographic early pregnancy diagnosis in cows (*Assiut-Egypt, 2000-2007*).

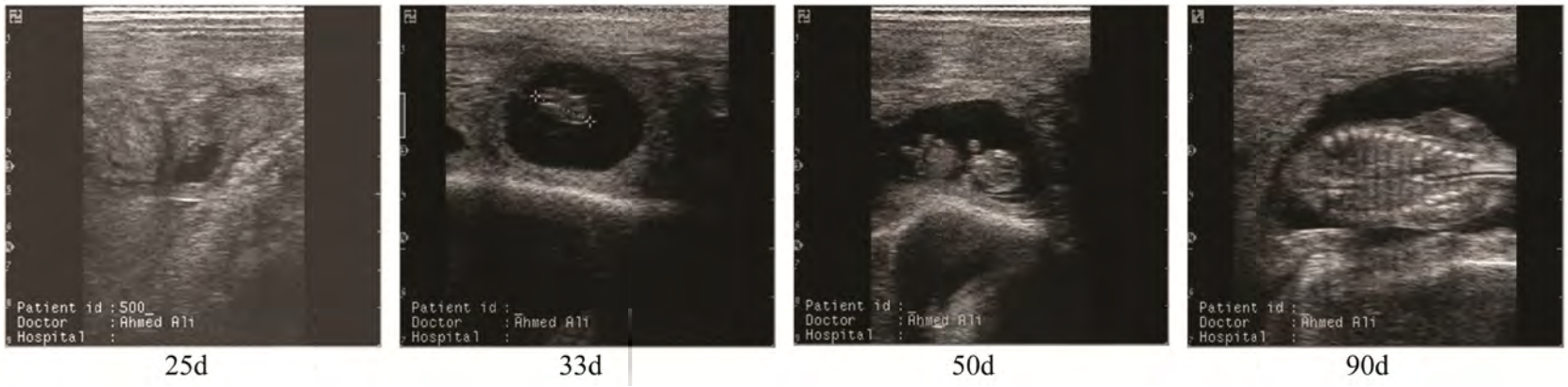


Fig. 43: Ultrasonographic early pregnancy diagnosis in buffalo-cows (*Assiut-Egypt, 2000-2007*).

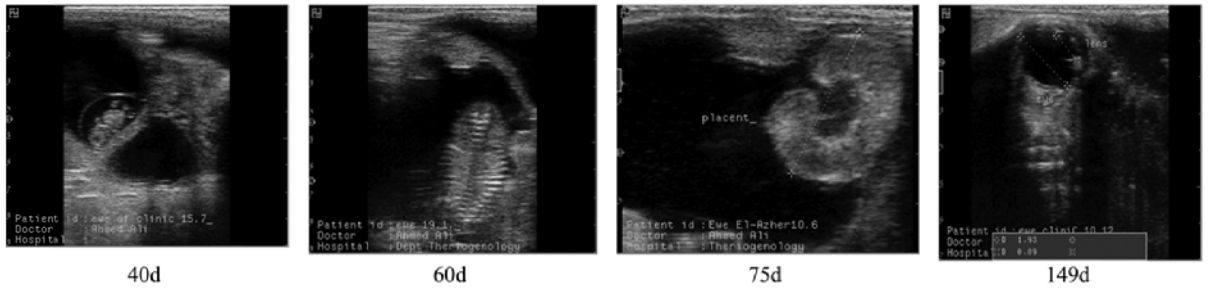


Fig. 44: Ultrasonographic early pregnancy diagnosis in ewes (*Assiut-Egypt, 2000-2007).*

Chapter 7

Complications of Pregnancy

Pregnancy failure is a term used to denote failure of a fertilized egg to develop into a newborn. The consequences of such a failure depend largely on the stage of pregnancy at which death of the conceptus occurs. Uterine torsion and vaginal prolapse are accidents of gestation. Other non-infectious complications of gestation are either fetal or placental in origin. Fetal mummification and maceration, hydrallantois and hydramnios as well as abortion will be discussed.

The Common diseases during pregnancy in farm animals are shown in Table (13) and illustrated in Figs. (45-52).

7.1. Disturbances during Pregnancy in Female Camels

7.1.1. Embryonic and fetal losses

In camels, calving rates rarely exceed 40% in nomadic herds and 70% in more intensive herds. In addition to low calving rates, camel herds suffer from high neonatal loss sometimes reaching epizootic proportions. In South American camelids, llamas and alpacas, birthing rates are slightly better, but high rates of pregnancy loss and infertility represent a major complaint in practice. In alpacas, the mean annual fertility reported is 50%, whereas in llamas the mean birth rate is 45.9%. Low fertility in alpacas seems to be due to the 50–57.8% reported embryo mortality up to 30 d of gestation.

Embryonic mortality, often associated with genetic causes, has been cited as a significant factor. Two or three CL in 13.65% and 1.22% of 491 single births was found, whereas the twinning rate was only 0.4%. Only one calf is produced by the camel. These observations clearly indicate the early occurrence of embryonic mortality. The causes of embryonic deaths include various pathological conditions such as metritis and pyometra, as well as genetic abnormalities resulting from inbreeding; hormonal disturbances and so on. In Tunisia, it was customary to service females with a sire from the herder's own colony, thereby increasing the level of inbreeding. One of the detrimental effects of inbreeding is the depression of low heritable characters such as fertility, reflected in increased abnormal germ cells and the early degeneration of zygotes and embryos. This abnormality is well known in bulls and other domestic animals, and there is little reason to doubt its occurrence in camels also. Prenatal deaths in many species are frequently followed by embryonic resorption and a return to estrus. However, since the camel has limited annual breeding period, the repeat-breeder camel would be unlikely to come back into season before the following year.

7.1.2. Uterine Torsion

Uterine torsion has been described in all camelids. Most of these torsions are clockwise (right) and can be complete (360°) or partial (90, 180 or 240°). This affection is considered by some authors as the leading cause of dystocia and cesarean section in *Camelidae*. The female is usually presented after unsuccessful and long first stage of labor, accompanied by signs of abdominal discomfort including frequent sitting and rising up, rolling and self-auscultation. The prognosis of uterine torsion is good for both the dam and fetus if diagnosed and treated early. There are two common stages of pregnancy at presentation: 8–10 months and at parturition. Clinical signs of uterine torsion are quite variable, ranging from mild discomfort to severe colic, diarrhea, and anorexia. Diagnosis is based on transrectal palpation of the broad ligaments and vaginal exploration. Clockwise torsion is indicated if the left broad ligament is stretched across midline to the right and over the uterus, whereas the right ligament is shorter and pulled ventrally and medially under the uterus. Palpation of the broad ligament may elicit a severe painful reaction. Diagnosis by vaginal palpation depended on the direction of vaginal folds. Direction of the torsion needs to be ascertained before attempting nonsurgical correction. Correction of uterine torsion can be accomplished nonsurgically by rolling or surgically. Rolling should be considered only if the uterus and its vasculature are not compromised. Rolling may be performed under general anesthesia, sedation, or without sedation. The female is placed on lateral recumbency on the side of the direction of the torsion and rolled while the fetus is maintained in position with a small plank or with the fists. Surgical correction may be performed following flank or midline laparotomy. Midline laparotomy is the preferred method in late pregnancy. The success rate of both rolling and surgical correction is very high, as is survival of the fetus. Complications of uterine torsion include uterine rupture/hemorrhage, endotoxemia, and death of the dam. Uterine rupture is often secondary to severe or inadequate clinical management of a uterine torsion.

7.1.3. Vaginal prolapse

Vaginal prolapse occurs during the last 2 to 3 months of pregnancy. It is probably due to softening of the tissue resulting from increased estrogen concentration during the last part of pregnancy. Most of vaginal prolapses concern older females and females in very good body condition. The prolapsed vagina is sometimes limited and visible only when the female is in the sitting position. However, with increased inflammation and edema of the tissue, the prolapsed part of the vagina increases in size and becomes permanently exteriorized. Prolonged periods of prolapse lead to increased inflammation of the prolapsed tissue and even severe necrosis of the vaginal mucosa. The prognosis for the life of the fetus and dam is relatively good if the condition is treated early. Treatment consists of replacing the prolapsed part of tissue after cleaning it with a mild antiseptic solution or physiological saline and maintaining it in place with a vulvar suture using Gerlach needle and gauze or by Buhner method. In the alpaca and llama, a shoelace suture pattern is sufficient. More advanced cases of prolapsed vagina with increased tenesmus may require epidural anesthesia. The animal should be monitored regularly and the suture removed if signs of impending parturition are observed.

7.1.4. Fetal Maceration

The entire fetus may still be present in the uterus in some cases but in other cases only the fetal bones are detected by rectal palpation, ultrasonography. Maceration could be due to an incomplete abortion where the fetus fails to be expulsed because of the lack of uterine contractions or insufficient cervical opening. The uterine cavity may become infected leading to an autolysis of the fetus. No signs of illness are usually observed.

7.1.6. Abortion

In the dromedary, reported abortion rates range from 2 to 11%. Various infectious, traumatic or toxic factors have been associated with abortion in *Camelidae*. Suspected causes of abortion include non-specific uterine infections, brucellosis, toxoplasmosis, trypanosomiasis, twining as well as stress and abdominal trauma. Abortion in the female dromedary has been associated with hemorrhagic disease and severe hemorrhagic necrotizing placentitis accompanied by edema. Abortion in llamas and alpacas has been associated with toxoplasmosis, *Chlamydia* and *Piroplasma*. Iatrogenic abortion can occur following administration of prostaglandin F2 alpha or its analogues or anti-inflammatory steroids. The involvement of Brucella infection in causing abortion was investigated in a breeding female subpopulation of 756 camels. The results of this study suggested that Brucella infections did not contribute significantly to abortion in camels.

An outbreak of abortions and high neonatal mortality has been attributable to Trypanosoma evansi infection in camels. A total of 16 females were diagnosed, 2 of which showed moderate signs of chronic form, particularly hyporexia and intolerance to exercise. The main laboratorial findings were regenerative anemia (hemolytic anemia), lymphocytic and monocytic leukocytosis, hyperproteinemia, hyperglobulinemia, hypoglycemia, serum urea increased, and serum iron decreased. The most characteristic finding in the examined females would be the uremia, probably due to the higher protein metabolism. Trypanosoma evansi was diagnosed for the first time in the Canary Islands (Spain) in 1998 in a dromedary camel. Seroprevalences of 4.8% up to 9% have been observed using different diagnostic methods. Affected animals have been treated but the dissemination of the disease is unknown. This article presents an outbreak of abortions and high neonatal mortality attributable to T. evansi infection in camels as well as the clinical assessment of the affected animals. The patients were diagnosed by routine checking (three pregnant animals), after abortion (five dams), or after delivered premature or weak calves (eight dams). At clinical examination, 2 out of 16 affected animals showed moderate signs of chronic form, particularly hyporexia and intolerance to exercise. The aborted fetuses were aged 6–8 months of gestation, approximately.

7.2. Disturbances during Pregnancy in Mares

7.2.1. Pregnancy failure

7.2.1.1. Failure between 1 and 5 days

Undoubtedly, some fertilized eggs fail to develop further and die in the uterine tube. As in the case for unfertilized eggs, these never reach the uterus. The percentage of such failure is unknown. The mare has a normal oestrous cycle unless other events cause complications.

7.2.1.2. Failure between 5 and 15 days

Fertilized eggs enter the uterus about 5 or 6 days after ovulation. Failure to develop further may be due to several causes, but those known are: Endometritis not only produces an environment (inflammatory) unlikely to support pregnancy development, but also initiates premature lysis of the CL; Recent ultrasound studies have shown that some mares pregnant at 10–12 days are no longer pregnant at 17–18 days; this suggests failure of maternal recognition of pregnancy. The corpus luteum therefore regresses at the normal time or prematurely; cyclic oestrous behavior is obviously resumed, usually at the normal interval, although mares may ‘short-cycle’.

7.2.1.3. Failure between 15 and 36 days (pseudopregnancy type I)

If the mare has recognized that she is pregnant after 14–15 days, the CL (corpus luteum verum) persists and the mare does not return to heat. Clinically, all the features associated with pregnancy develop in the tubular genitalia. Pregnancy failure results in resorption. Ultrasound imaging shows an initial reduction in volume of the conceptus (small size for age) followed by increased echogenicity of the fluids, inward bulging of the uterine wall and, finally, loss of the normal appearance. Palpably, the pregnancy bulge disappears due to dehydration, but: All the other features of early pregnancy (closed cervix, tonic uterus, persistent CL, follicular growth) persist; the CL, which is responsible for those features (but cannot be palpated), may last for 2–4 months; this is pseudopregnancy type I. Natural demise of the CL, or its premature lysis by exogenous prostaglandin will cause rapid return to oestrus and expulsion of remnants of pregnancy. The induced heat should be as fertile as any other, but in practice it is less fertile, particularly in pregnancies that have survived for more than 20 days.

7.2.1.4. Failure between 37 and 140 days (pseudopregnancy type II)

During this period, eCG (PMSG) is normally produced. eCG complicates the endocrinological environment so that pregnancy failure during this period results in continued production of eCG until the endometrial cups regress naturally (there is no known method of accelerating their demise). The resulting syndrome is known as pseudopregnancy type II. For as long as eCG is produced, the mare will not get in foal again. Two patterns of reproductive behaviour have been described during pseudopregnancy type II: (1) In ponies and some Thoroughbreds, recurrent periods of oestrus occur with follicular development, but follicles become luteinized (i.e. produce progesterone) without

ovulating. (2) In some Thoroughbreds, the ovaries become small and quiescent and the mare enters a period of 'anoestrus'.

7.2.1.5. Failure from 140 days to term

The time at which this period begins and the previous one ends is very variable, because of the individual differences in the length of time that eCG remains in the circulation (60–200 days). Fetal death after eCG disappears from the blood is characterized by abortion because: hormonal control of pregnancy at this stage is exercised by the fetus and placenta; fetal death is followed by a rapid decrease in circulating estrogen and cessation of progesterone production; these changes cause cervical dilation and increased myometrial contractions which ensure expulsion of the fetus and usually also the membranes. Because of the ease of abortion, mares rarely show signs of malaise and often abort unnoticed. After abortion, the mare will come back into heat quite rapidly Fertility after abortion should be good, but retained placenta may be more common than after normal birth; bacterial cause of abortion may leave residual infection which requires resolution before conception can occur; abortion of twins may delay subsequent conception, presumably because of over distension of both uterine horns. Rarely, possibly because of cervical stenosis, abortion is incomplete and fetal maceration occurs.

Equine herpesvirus (EHV), or rhinopneumonitis virus, causes abortion in mares (especially sub-type 1). The virus also causes respiratory disease; this is most noticeable in horses (foals and yearlings) which are exposed to the virus for the first time. It may also cause paresis with ataxia, tail flaccidity and urine dribbling, or a fatal paralysis. The sources of the virus are: clinically affected animals; nasal secretions contain virus in animals which are both obviously infected and those which fail to show clinical signs; Aborted fetuses and their membranes; infected foals which are born live at term but which shed virus for the first week of life; mares which have aborted; these shed virus from the genital organs for only a short period. Keep weaned foals and yearlings away from pregnant mares. Non-pregnant mares and stallions are vaccinated annually. These regimes reduce the incidence of abortion; (2) *Killed bivalent vaccine:* This is given in three doses, one month apart, with subsequent boosters every six months. This regime is effective at controlling abortion and respiratory disease; (3) *Modified live virus vaccine:* This is not given to pregnant mares, but does provide good immunity. Vaccination should be considered where the cost relates favourably to the potential value of the foals at risk. Vaccination frequency is under review, but should be as stated by the manufacturer. Ideally, all horses on premises should be vaccinated.

Equine viral arteritis causes abortion in mares. EVA is a notifiable. EVA is venereally transmitted, as well as being transmitted via the respiratory tract. The virus causes a wide range of clinical signs other than abortion, including conjunctivitis (pink eye), cough, dyspnoea, diarrhoea, colic and subcutaneous oedema. In the stallion there may be scrotal and preputial oedema. The severity may vary from slight pyrexia with conjunctivitis to severe illness. The sources of the virus are: clinically affected animals, both mares and stallions, via nasal secretions (droplet infection); aborted fetuses and their membranes; genital organs secretions for up to three weeks after abortion; infected semen (including chilled and frozen-thawed semen). The majority of stallions are infective only for a short period of time. There are no specific gross lesions. The fetus may be autolytic and

histologically there may be necrosis in small arteries. The placenta may be autolytic. Virus may be isolated from nasal secretions, aborted material and semen. There may be rising antibody titres; antibodies develop 1–2 weeks after infection.

Because EVA is usually only excreted for three weeks, a quarantine period exceeding this time should be considered. Screening of stallions for carrier status (antibody titre) is essential.

A number of other infectious causes of abortion exist in certain countries and include: Equine infectious anaemia; Piroplasmosis; Leptospirosis; Dourine. When the cause of an abortion is not investigated, it is easy to implicate some previous event, e.g. thunderstorm, kick by another horse, change in management, excess exercise, vaccination, worming, etc. In most cases, these are merely incidental events, but being able to apportion blame is an understandable desire for the disappointed mare owner. Running the mare with a gelding does not cause abortion unless the gelding is 'riggy' and continually mates with the mare. Some less common causes of abortion are: twisting of the umbilical cord: when the cord is tightly wrapped around the trunk or a hind limb it is probable that the circulation may be impeded sufficiently to cause fetal death: twisting the cord on itself may be the result of fetal distress during an abortion, or may directly cause fetal death over a long period; evidence of previous (non-fatal) umbilical torsion is often seen when the urachus (thin walled) is dilated due to accumulation of urine or the bladder is grossly distended; fetal abnormalities, e.g. hydrocephalus, occasionally stimulate abortion; chronic endometrial change reducing functional placental area sufficiently to cause fatal malnutrition of fetus; development of excess fetal fluid is very rare and may either stimulate abortion or necessitate therapeutic termination of pregnancy; iatrogenic abortion is rare: drugs which are known to upset pregnancies in other animals are unlikely to be used in sufficient quantities in mares in late pregnancy (e.g. oxytocin, prostaglandin, xylazine, etc.); Severe malnutrition at 20–30 days may cause resorption. Other Abnormal Events during Pregnancy

Sporadic abortion may occur due to ascending infection with *Aspergillus*. Most abortions occur at approximately ten months' gestation. The fetus is often small and emaciated and may be expelled alive. Commonly there is extensive chronic placentitis. The membranes are often oedematous and have typical necrotic plaques. Sometimes, grey nodules are found in fetal lungs, and, rarely, on the skin. Culture will reveal hyphae within placental lesions and fetal stomach contents.

Premature and dysmature

Foals born before 320 days of gestation are arbitrarily defined as premature, although many of these survive; foals born after 320 days are sometimes weak and appear unprepared for extrauterine life; these are said to be *dysmature*. In some mares, apparently fully-mature foals are born at approximately 320 days. Pregnancy length is variable and is shorter for foals born in summer and for pony mares. Causes of Pregnancy Failure

7.2.2. Twining

Twin pregnancies pose a problem because two fetuses are trying to develop with a placental attachment area designed for one. In early pregnancy there appears to be a mechanism for causing death of the smaller of twins in some cases; this reduces the scale of later problems. If twins persist as

pregnancy advances, the nutritional requirement of the fetuses increases, fetal growth is limited by placental attachment area and there are three common outcomes: (1) One fetus becomes larger than the other, the smaller, emaciated fetus dies and usually both are aborted at 8–9 months of gestation. This is the most common outcome (80% of cases); (2) The fetuses are similar in size, go to term and two small, weak, sickly foals are delivered. These may die or have to be destroyed. (3) The size difference between the fetuses is large and the smaller fetus dies early in the pregnancy and is mummified. The larger twin is normally born alive and is able to survive.

An obvious advantage of the scanner in veterinary stud routine is the ability to detect a worthwhile proportion of twins at a much earlier stage after mating than is possible by rectal palpation alone and hence when there is still a period of time available to remedy the situation before the development of the horse CG-secreting endometrial cups at around Day 36 after ovulation. In mares which have twin conceptuses situated in separate uterine horns, the treatment of crushing one conceptus manually without harming the normal development of the contralateral conceptus is relatively simple to perform and is likely to have a high rate of success if carried out before Day 30 after ovulation. This is certainly not the case when attempting to crush one of twin conceptuses after about Day 35; the manipulation is much more difficult to perform at this later stage of pregnancy and in the majority of cases the other conceptus is also resorbed. It is obviously not possible to crush selectively one of very close conceptuses and the preferred treatment in such animals is the induction of abortion and luteolysis by treatment with prostaglandin so that the mare can return to estrus and be mated again as soon as possible.

7.2.3. Premature placental separation

Premature placental separation occurs most commonly at parturition. The foal is normally hypoxic and is born weak (‘dummy foal’) or stillborn. Velvet-like, red, chorionic membranes bulge through the vulval lips at term. The chorioallantois should be ruptured, and the foal delivered as soon as possible. Resuscitation facilities should be prepared for the foal. Supplemental oxygen may be necessary even in the foal that initially appears normal.

7.2.4. Ruptured pre-pubic tendon or abdominal wall rupture

This occurs mainly in Shire and heavy horses in late pregnancy. Mares are often aged. It may occur in mares with hydrallantois, twins or a single large fetus. It is characterized by massive ventral swelling edema, abdominal pain and often recumbency. The mare often develops a ‘saw-horse’ stance, with hind and forelegs extended. Prognosis is poor, although live foals may be produced by assisted delivery after parturition induction. Traction is usually required at natural foaling, since the mare is unable to produce effective abdominal contractions.

7.2.5. Uterine torsion

Uterine torsion can occur at any time during late pregnancy, but is most common during mid to late term. The cause is speculative, possibly following a fall. The uterus twists about its long axis to 90–360°. If the torsion is sufficient, it will restrict blood flow and the uterus will become congested and friable. The mare shows signs of moderate to severe colic. Diagnosis is by examination *per*

rectum; if the twist is anti-clockwise, the right broad ligament can be felt stretched to the left over the dorsal surface of the uterus, and vice versa. Most torsion occurs cranial to the cervix and therefore can only rarely be diagnosed by vaginal palpation. The prognosis for the foal is poor because of interference with the blood supply to the uterus (due to compression of the major vessels). The prognosis for the mare depends on the speed of diagnosis and treatment. Treatment is either: correction of the torsion via ventral laparotomy under general anaesthesia. If this is done in late gestation, the pregnant mare may abort, but some will foal normally at term; correction of the torsion via standing flank laparotomy; anaesthetising and rolling the mare. Reduction of the torsion close to term is best followed by Caesarean operation via the same laparotomy.

7.2.6. Fetal mummification

Once a fetus has acquired a recognizable skeleton (after four months) the bones will remain intact and the dehydrated fetus, a mummy, remains recognizable. This situation only occurs in mares in which one of twins has died, because death of a single foal causes abortion unless the fetus becomes lodged in the cervix. Mummification therefore depends upon persistence of the corpus luteum.

7.2.7. Hydrops of the fetal membranes

Excessive fetal fluid may develop within the fetal membranes, although this is rare, causing: (1) Hydrops amnion; (2) Hydrops allantois. Hydrops allantois is the most common, and is usually seen after seven months of gestation; 100–200 l of fluid may accumulate. Clinical signs include swollen abdomen and laboured breathing. The fetus is often non-viable and abortion may occur. Abortion should be induced if it is not spontaneous; drainage of fluid and manual extraction is most appropriate. The sudden loss of fluid may result in severe shock and even death. Pre-treatment of the mare with fluids and corticosteroids is prudent. The condition does not necessarily recur at the next pregnancy.

7.2.8. Prolonged gestation

Gestation may extend to 310–370 days. Most mares that do not foal at the normal time are healthy, and the owner should be reassured that this is probably normal.

7.3. Disturbances during Pregnancy in Cows

7.3.1. Uterine torsion

Uterine torsion usually occurs near term and is usually found at parturition because of the subsequent dystocia. The attachments of uterus and manner in which cows rise are assumed to play a role in the development of this condition. Diagnosis can be made by 1) manual vaginal exam, the vaginal wall can be felt to be twisted or spiraled. This can be visualized with the aid of a speculum; 2) By rectal exam, the broad ligaments can be felt to be crossed and the uterus twisted. The vulva can often be seen to have a slight twist of the dorsal portion. It is important to determine the direction of the torsion during the examination. Before and during correction, insure that things are going in the

proper direction. If the cervix is open, a detorsion rod may be used. Alternatively, the torsion may be corrected manually by rocking the fetus until enough momentum is achieved to flip the uterus. If the cervix is closed other methods must be employed such as the "Plank in flank" method or surgical (C-section) correction. In plank method, the dam should be casted on the same side of torsion on a slopped and rough ground with head kept down. A rough plank is placed on the dam flank while a moderate person (60 kg) stands or sits on it to fix the uterus. The dam is rolled to the other side slowly. The operator may help by fixing the cervix or any fetal part through vagina.

7.3.2. Vaginal prolapse

This is often a chronic condition and is hereditary. There is a breed predisposition with Hereford. Vaginal prolapses often recur. For this reason, it is recommended to cull cattle with a vaginal prolapse. They usually occur prepartum, in late gestation, when the cow is under the influence of rising estrogen and experiencing relaxation of the tissues. They may occur postpartum or may be associated with follicular cysts. Upon presentation, the vagina, with or without the cervix, is seen protruding from the vulva. The tissue is often dry and necrotic. Tenesmus is common. When considering the prognosis, although rarely life threatening, consider the chance of recurrence and inheritance and recommend culling. These are much easier to replace but harder to maintain in the correct position. Correction is aided and tenesmus reduced with an epidural anaethsia. The tissue is cleaned,, lubricated and replaced. After replacing the prolapse, the cow may be maintained so that her hind quarters are elevated (dairy cow). These need to be sutured to help reduce the risk of reoccurrence. There are numerous methods of fixation. Probably one of the most effective is the Bühner stitch. This requires a Bühner needle and Bühner tape. The Bühner stitch must be removed prior to parturition. Modified Caslick's are also used occasionally with varying degrees of success. Pessaries are also used occasionally with varying degrees of success.

7.3.3. Fetal mummification

This occurs in cases of fetal death without involution of the corpus luteum and fetal expulsion, followed by autolytic changes, absorption of the fetal fluids and involution of the placenta. In cows the maternal caruncle involutes and hemorrhage occurs between the placenta and the endometrium, leaving a reddish-brown, gummy mass that imparts a reddish brown color to the mummified fetus. The etiology is varied and ranges from infectious causes such as BVD, leptospirosis, etc. to non-infectious causes such as genetic, compressed umbilical cord, etc. Diagnosis is based on the presence of a CL, the lack of fremitus in the uterine artery and lack of fetal fluid in the uterus. The fetus feels dry and mummy-like on palpation. Oftentimes the head, ribs, etc. can be felt. Prognosis is good if the fetus is removed. After the fetus is removed, conception usually occurs 1-3 months later. Treatment is accomplished by administering PGF2α to lyse the CL. Steroids are ineffective with dead fetus and non-functioning placenta. After treatment, check the vagina because sometimes the mummy may be lodged in the vagina when expelled.

7.3.4. Fetal Maceration

Fetal maceration results from death of the fetus followed by dilation of the cervix and incomplete abortion or dystocia, usually during the last half of gestation. This condition can be due to a variety of miscellaneous organisms. On palpation per rectum, the uterine wall is thick, little or no fluid is present in the uterus and you may be able to palpate fetal bones and pus, or bones crepitating against each other in the uterus. The prognosis is poor for cows with this condition. This is not a "retained CL" problem so lysis of the CL is not helpful. Endometrial damage is present even if all fetal parts are removed. Treatment is very difficult. The cervix cannot usually be dilated sufficiently to remove all the fetal parts and any remaining fetal parts act as an intra-uterine device. Surgery has been performed in valuable individuals but is very difficult.

7.3.5. Hydrallantois and hydramnios

Hydrallantois, or hydrops of the allantois, is due to a defective placenta (the chorio-allantois). The fetus is normal. The condition is characterized by a rapid accumulation of watery, clear fluid, usually in the last trimester. The cow is rounded in the caudal view, and you normally can't palpate the fetus or placentomes. Usually the condition results in a sick cow with anorexia, decreased rumen motility, dehydration and weakness. The cow may be down. The placenta is thick. If the cow survives, postpartum metritis is common. The condition usually ends in death or intervention. The prognosis is guarded to poor for life and fertility. Treatment consists of Caesarian section with a slow drainage of fluid and perioperative support. Dexamethasone can be used if the cow is not down.

Hydramnios, or hydrops amnios, is due to a defective calf, usually attributed at least partly to a defect in swallowing. The placenta is normal. The condition is characterized by a gradual accumulation of thick, viscid fluid during the last half of gestation. The cow has a pear shaped caudal view. Usually you can palpate the fetus and placentomes. The cow is clinically otherwise unaffected. The pregnancy usually goes to term, and frequently a small, deformed fetus is delivered. Postpartum metritis is uncommon. The prognosis is good for life and fertility. No treatment is required. The cow may be allowed to go to term or induced to calve.

7.3.6. Abortion

Abortion in the cow is defined as foetal death and expulsion between day 45 and day 265 of pregnancy. Most cattle herds suffer an abortion rate of 1-2%. A single abortion is thus no great cause for alarm. An annual abortion rate up to 5% is considered to be normal. This figure excludes most abortions occurring during the second and third month of gestation as these often go undetected. An abortion rate in excess of 10% is considered an abortion storm. Causes of abortion: 1) Non-infectious causes: genetic; environmental: temperature; nutritional: phytotoxins including mycotoxins; iatrogenic: administration of abortifacient drugs; 2) Infectious causes: general infections with high fever; specific infections such as brucellosis, BVD etc.

For control and prevention of abortions the following are important: 1) Proper hygienic and biosecurity measures in the cow's environment and feed storage ; 2) Isolation of aborting cows and immediate removal of aborted materials; 3) Systematic evaluation of the feed for mycotoxins and

other phytotoxins; 4) Adequate immunization against infectious diseases causing abortion; 5) Maintenance of adequate breeding and treatment records to avoid insemination of pregnant cows and administration of drugs that may cause abortion to pregnant cows; 6) Well-kept records can be very useful in the investigation of an abortion problem. Breeding dates, parity, production information and health events (for example, disease or vaccination) can all help to identify factors which may be associated with the abortions. Other 'herd level' information such as ration changes, new additions, personnel changes, etc., should also be recorded. This kind of information should be kept in a readily accessible format on all dairy farms and will serve many functions in addition to being useful for investigating abortion problems; 7) Sample collecting which will be used to diagnose the cause of an abortion(s), the principle of "*the more the better*" should be followed. Ideally, the whole fetus and placenta should be saved and placed in a clean bag, which should then be refrigerated as soon as possible. In some situations, paired blood samples may also help to diagnose an active infection in the cow, such as BVD or leptospirosis. The first sample should be taken as soon as possible after the abortion is noted, with the second sample being collected in 2 - 4 weeks time. While this does require some more effort than collecting only a single blood sample, the results will generally be more meaningful.

7.4. Disturbances during Pregnancy Buffalo-Cows

7.4.1. Uterine torsion

See causes of dystocia in buffalo-cows.

7.4.2. Vaginal prolapse

Vaginal prolapse is a commonly encountered problem in pregnant dairy buffaloes, particularly during the last quarter of gestation.

7.4.3. Abortion

Bacteria and viruses can cause abortion in buffaloes. The abortigenic agents in buffaloes are: *Brucella spp*., *Trueperella pyogenes*, *Chlamydophila spp*., *Coxiella burnetii*, *Bacillus licheniformis*, *E.coli*, *Leptospira spp*., Bubaline Herpes Virus-1 (BuHV-1), Bovine Viral Diarrhoea Virus.

Brucellosis of the buffalo is caused by *Brucella abortus* and, increasingly frequently, by *Brucella melitensis.* The ability of brucellae to survive in the external environment makes them difficult to eradicate from herds. The infection is contracted orally, through contaminated feed or water, or, more rarely, through genital, mucosal or transcutaneous routes. Abortion generally occurs between the sixth and ninth months of gestation, and is often accompanied by retention of the placenta. When the infection is contracted in the final stages of gestation, it may cause premature delivery and neonatal death. The calves that survive may remain infected and abort during their first pregnancy, thus giving rise to a cyclical return of the infection. The microbiological diagnosis is based on bacteriological examination of the cotyledons and of the main foetal organs. Molecular biology

techniques are now available which can identify the biovar involved. In areas where the disease is endemic, eradication may be favoured by the use of the RB 51 vaccine; experimentation on buffaloes has demonstrated that this vaccine is harmless, elicits good immunity and is not eliminated in milk.

Chlamydiae are members of the family *Chlamydiaceae*, a group of obligate intracellular bacteria. Ruminants can be infected by two species: *Chlamydophila abortus* and *Chlamydophila pecorum. Chlamydophila abortus* causes abortion in small ruminants. This pathogen is also deemed to be responsible for abortion in buffaloes. Abortion, which may even become epidemic, occurs in the second half of pregnancy. The fetus may present haemorrhagic petechiae on the myocardium and dense, pale yellow, mucous content of the abomasum. In the positive cases, macroscopic lesions of the placenta have never been recorded. The diagnosis is carried out by means of immunochromatographic testing or PCR. *Chlamydophila pecorum* has long been recognized as the aetiological agent of encephalomyelitis in buffalo calves. Recent studies conducted by means of molecular biology techniques on positive fetal tissues from archives have enabled the species involved to be typed as *pecorum*. It can therefore now be claimed that *Chlamydophila pecorum* is the main agent responsible for abortion in buffalo cows, as well as for encephalomyelitis.

Abortion caused by *Trueperella pyogenes* is extremely frequent among buffalo cows, though epidemic outbreaks have not been reported. Abortion generally occurs in the final phase of gestation, and may be followed by retention of the placenta, endometritis and metritis. In the buffalo, this micro-organism may also cause mastitis, omphalitis, pulmonitis and septicaemia. The diagnosis is based on bacteriological examination of the organs. *Trueperella pyogenes* develops in 24-48 hours, forming haemolytic colonies on agar supplemented with 5% sheep erythrocytes. Biochemical tests yield definitive microbial identification

E. coli produce heat-stable toxins, verocytotoxins and necrotizing cytotoxic factor. The buffalo is an important reservoir of verocytotoxic *E. coli* serotypes, especially O157. *E. coli* may also cause abortion, albeit sporadically. To date, it is not certain whether abortion is caused by the bacterium and its structural antigens or by the cytolytic action of its toxins. The diagnosis is based on the serological examination of the fetal organs. *E. coli* develops in MacConkey agar medium, fermenting lactose and producing reddish-pink colonies. Any haemolytic activity can be evaluated by means of blood agar. PCR is a useful tool in detecting, from isolated strains, the gene sequences responsible for coding virulence factors or toxins.

Serological studies and the sporadic isolations described in the literature seem to suggest that various serotypes of *Leptospira* spp. are present in many buffalo herds. Research has led to the detection of the bacterial genome belonging especially to the *pomona*, *canicola* and *hardjo* serotypes in several fetal buffalo kidneys. These reports, though as yet partial, seem to point to a possible role of *Leptospira* spp. in causing abortion in buffalo cows.

Coxiella burnetii is one of the possible aetiological agents responsible for abortion in buffalo cows. This species may therefore also be responsible for transmission of the infection to humans.

During the course of bacteriological investigations carried out on aborted buffalo fetuses, several other bacteria may be isolated, such as *Pasteurella* spp. *Pseudomonas aeruginosa, Staphilococcus* spp. and *Streptococcus* spp.

7.5. Disturbances during Pregnancy in Ewes and Does

7.5.1. Vaginal prolapse

Vaginal prolapse *ante partum* is a well-known disease affecting sheep and goats during the last trimester of gestation. The exact cause of this frequently occurring prepartum disorder in ewes has not yet been identified. Dependent from the flock, the incidence varies from 0.1% to more than 15%. Peripheral concentrations of progesterone and estradiol-17β seem to have no influence on the occurrence of vaginal prolapse in ewes. Prolapses in pregnant does usually happen during the final 30 days of pregnancy. Rectal prolapses appear in does that have been improperly fed and allowed to become too fat. Proper nutritional management makes rectal prolapses unlikely to occur. Vaginal prolapses are mostly hereditary and can be bred out by mating the doe with an unrelated buck whose previous female offspring have not prolapsed. Does that prolapse more than once should be culled from the herd.

Returning a prolapse to the inside of the ewe's and goat's body must be done very carefully. To prevent infection, clean the prolapse with a sterile solution. Apply water-soluable lubricant to the gloved hand being used to re-position the prolapse. Using the flat palm of the gloved hand; press gently and with even pressure on the prolapse back inside the animal. This is a two-person job; one person has to hold the animal in a standing position while lifting its rear legs off the ground so that it can't push against the hand of the second person, who is attempting to return the prolapsed organ back inside the goat. Sometimes it is necessary to place the animal in inclined position with the head down to get the proper angle that allows reinsertion of the prolapse. If the prolapse has been outside the body for several hours or overnight, causing it to dry out and therefore become more difficult to put back inside the animal, tannic acid, alum or granulated sugar can be sprinkled over the cleansed prolapse. These helps shrink the prolapse, easing its return inside the goat's body. If none of these procedures is successful, it is time to call a veterinarian. Once the operator gets the prolapse back in place, he can install a series of purse string stitches to hold the prolapsed organ inside. The precise lambing date must either be known or the producer must frequently check the animal for signs of labor. When the dam's water breaks, the stitches must be cut immediately so that her kids can be born. Otherwise they will drown.

7.5.2. Pregnancy toxemia

Pregnancy toxemia is a metabolic disorder caused by low glucose concentrations in the blood and excessive breakdown of body fat to compensate. "Ketones" are the toxic by-product produced during this rapid breakdown of fat, and it is possible to test for their presence in the ewe's urine. Inadequate nutrition during the last one-third of pregnancy is the primary cause of low blood sugar/pregnancy toxemia, as ewes cannot consume enough feed (energy) to meet the demands of their

growing fetus(es). This is because approximately 70 percent of fetal growth occurs during the last 4 to 6 weeks of pregnancy. Over-conditioned (condition score 4/5 or more) ewes are susceptible to pregnancy toxemia because of fat in their abdominal region – there simply isn't enough room in the gut for the ewe to eat enough - and excessive fat resources for breakdown. Under-conditioned (condition score 2 or less) ewes are susceptible because they cannot eat enough to meet their own nutritional needs, let alone the added burden of developing fetuses. Ewes carrying multiple births are also at high risk for pregnancy toxemia. Ewes carrying twins require 1.9 times the dry matter intake as ewes with singles. Ewes with triplet fetuses require 230% more energy than ewes with singles. In fact, anything that affects the ewe's ability to eat enough during late gestation can result in pregnancy toxemia: multiple fetuses, fat ewes, thin ewes, small ewes, timid ewes, granny ewes, dental disease, parasitism, and lack of exercise. The symptoms of pregnancy toxemia are vague and can be similar to other diseases, especially hypocalcemia (or milk fever). Milk fever can be differentiated from pregnancy toxemia by the affected ewe's response to calcium therapy. Ewes in early stages of pregnancy toxemia will go off feed and appear lethargic. Their heads droop and they lag behind the rest of the flock and walk aimlessly. Teeth grinding and twitching is common. Eventually, affected ewes become depressed, weak and have poor muscle control. In latter stages, they lie down and are unable to rise. If left untreated, coma and death result. Successful treatment of pregnancy toxemia requires early detection and steps to quickly meet the energy (glucose) needs of the affected ewe. The most common treatment is to drench ewes with 40-50 mL of propylene glycol 2 to 3 times daily. Yogurt mixed with water will also provide energy and bacteria to stimulate the rumen. Intravenous glucose is another possibility, but harder for producers to do on the farm. Force feeding and/or injections of multiple B vitamins can help stimulate the ewe's appetite. Antibiotics can be administered to prevent pneumonia. In advanced cases, a caesarian section may need to be performed to remove the fetuses and save the ewe's life. If the lambs are near term can be saved. The nutrition of the entire flock should be suspect anytime a ewe shows indications of pregnancy toxemia. Of course, like other diseases, it makes more sense to prevent pregnancy toxemia than to treat it. To achieve this, it is absolutely essential that ewes be provided adequate energy in their ration during the last 4 to 6 weeks of gestation. Good quality hay should be provided, along with grain supplementation. Grain and molasses are excellent sources of energy. Hay alone usually doesn't provide enough energy for ewes carrying twins and triplets. Exercise is also deemed important in the prevention of pregnancy toxemia. Abrupt feed changes must also be avoided, and ewes should not be stressed during late pregnancy. There must be adequate feeder space so that all ewes can fit around the feeders and get their fair share of hay and grain. Producers should strive to have ewes in moderate body flesh (condition score of 3+) prior to lambing. Ewes should be prevented from becoming obese during early pregnancy, and thin ewes should be separated and receive extra feed until they achieve the desired condition score.

7.5.3. Abortion

It appears normal for about 1.5 to 2.0% (up to 5%) of the ewes in a flock to abort. Abortion rates significantly above this level cut into profit potentials, as what may start out as a few isolated cases can quickly escalate into an abortion "storm," resulting in 20-30% percent abortions or as high as 80% lamb mortality.

There are several infectious agents which are known to cause late-term abortions in small ruminants. The most common are *Campylobacter fetus* (also called Vibrio), *Chlamydia psittaci* (also called EAE or Enzootic Abortion in Ewes) and *Toxoplasma gondii*. Less common causes include Leptospirosis, *Brucella ovis* (related to epididymitis in rams), Q-fever, Border disease (related to BVD in cattle) and Bluetongue virus. Non-infectious causes of abortion include rough handling, fighting among animals, inadequate nutrition, and plant poisons.

Ewes infected with *Vibrio* typically abort during the last 6 to 8 weeks of pregnancy or give birth to weak or dead lambs. Once the ewe aborts, she is immune to the disease. *Vibrio* abortions are usually introduced into a flock via a carrier animal. A carrier is an animal that has aborted, but carries the infectious bacteria. It is important to note that the bacterium that causes Vibrio in cattle is different from the one that causes it in sheep.

Chlamydia abortions also occur during late pregnancy and may result in stillborn or weak lambs that die shortly after birth. *Chlamydia* is also associated with pinkeye, polyarthritis and pneumonia. The spread of enzootic abortion is believed to be primarily through contact with infected fetuses, placenta or vaginal discharges; though there is evidence to suggest that some "carrier" ewes may constantly shed the organism in their feces or from their lungs.

Vaccines are available for both *Vibrio* and *Chlamydia*, often in the same injection. They are designed for use at the beginning of the breeding season. They are killed vaccines, thus two shots are required the first year. After a ewe has received her initial two-vaccination series, only a single vaccination needs to be given in subsequent years. Some large producers have achieved good results simply by vaccinating replacement ewes.

Toxoplasmosis is a common intestinal, protozoal infection in cats. It can cause abortion in sheep at any stage of pregnancy, depending upon the stage during which the ewe was infected. It is generally accepted that ewes become infected when they ingest feed or water which has been contaminated with infected cat feces. The best prevention is to control cat populations by keeping cats away from pregnant ewes and/or maintain a healthy adult cat population and to prevent contamination of feed and water by nesting cats.

In order to develop a course of action and prevent abortion storms in subsequent years, the specific cause of abortion must be determined. Diagnosis is based on clinical signs and flock history, combined with laboratory diagnostics. It is crucial that the proper samples (both the fetal and placental tissues) be submitted to a veterinarian or state diagnostic laboratory in order to differentiate between abortion types. Tissues can be kept cold by packing in ice in a leak-proof, insulated container until they can be delivered. Blood sampling (before and after abortion) may also be warranted.

Controlling an abortion outbreak requires strict sanitation and separation of aborting ewes. Infected fetuses, placental tissues, and bedding must be properly disposed of (burned or buried). All aborting ewes or those with vaginal discharges should be immediately isolated from the main flock. Aborted ewes should not be used as foster mothers for female offspring, unless infectious causes of

abortion can be eliminated. Pregnant ewes should never be fed on the ground. Breeding stock from flocks that have experienced abortion storms should not be purchased.

Immediate vaccination and the use of antibiotics may help lessen losses during an outbreak. Ewes should be injected with an antibiotic (tetracyclines), and then started on a feed that contains antibiotics. Consult with a food animal veterinarian for an appropriate course of action and if using any drug extra-label.

It is important to note that humans are susceptible to many of the same abortion-causing agents as sheep. Care must be taken when assisting ewes during lambing and when caring of weak lambs. Plastic gloves and other protective clothing should be worn whenever contaminated material is handled. Unpasteurized milk or cheese should not be consumed. Pregnant women are especially susceptible to toxoplasmosis and should not handle infected cats or aborted fetuses.

Abortion caused by *Listeria monocytogenes* in ewes usually occurs in late gestation. There is some necrosis of cotyledons and the intercotyledonary areas, and the fetus is usually autolyzed. The fetal liver (and possibly lung) may have necrotic foci, 0.5-1 mm in diameter. Diagnosis is by culture

The major importance of *Brucella ovis* is as a cause of epididymitis in rams, but it also causes late-term abortions, stillbirths, and birth of weak lambs. *B melitensis* causes abortion in areas where it is found. *B abortus* occasionally causes abortion in sheep. *Brucella* abortions occur late in gestation, resulting in placentitis with edema and necrosis of the cotyledons and thickened, leathery intercotyledonary areas. Many fetuses aborted due to *B ovis* are alive at the beginning of parturition, although fetuses can be mummified or autolyzed. Most fetuses aborted due to *B melitensis* or *B abortus* are autolytic. Culture of the placenta, abomasal contents, and the dam's vaginal discharge are diagnostic. A vaccine for *B melitensis* is available in some countries. *B melitensis* and *B abortus* are zoonotic

Salmonella abortus ovis, *S dublin*, *S typhimurium*, and *S arizona* have caused abortions in sheep. *S abortus ovis* is endemic in England and Europe but has not been reported in the USA. The other serotypes occur worldwide. Most ewes are sick and febrile before aborting. There are no specific placental lesions, and the fetus is autolyzed. Diagnosis is by culture of placenta, fetus, or uterine discharge.

Bluetongue virus and Akabane virus (where present) cause abortion and congenital anomalies in sheep and are differential diagnoses for Cache Valley virus infection. *Coxiella burnetii* causes occasional abortion storms in sheep, with the clinical syndrome and fetal pathology being the same as for goats. *Neospora caninum* has been reported to cause occasional abortions in sheep with the lesions resembling those of *Toxoplasma gondii.*

What to do when abortion occurs?

- Never ignore abortions in sheep or goat herds. Conduct a thorough investigation immediately.
- Isolate the animal from the herd and keep it in a quarantine pen for further examination.

• Consider many different causes of abortion.

• Inform your veterinarian if you suspect infectious abortion in a goat herd; the veterinarian will refer you to a nearby diagnostic center.

• Consult the diagnostic laboratory prior to submitting your sample. The diagnostic center should be aware of the infectious agent most likely to be present in the area.

• To facilitate the diagnosis, keep detailed records and accurately identify each aborting animal and the stage of pregnancy at which the animal aborted.

• Refrigerate (avoid freezing) any fetus and placenta of an aborted kid to send to the diagnostic laboratory.

• Work with the local veterinarian to draw blood and to send serum samples from aborting does to the diagnostic laboratory for immunological tests.

• Consult your local veterinarian when you suspect infectious abortion in your herd. This might constitute a public health issue. Your veterinarian can guide you on the treatment and prevention procedure.

• Ask for performance and health records before purchasing new animals.

• Quarantine any new animals before introducing them into your existing herd.

• Be aware that certain classes of dewormers administered to pregnant does can cause insidious abortion or stillbirths, which can be mistaken as abortions caused by infectious agents.

• Be aware that certain poisonous plants can cause abortions in does. Identify plants in your area that can cause abortion and try to eliminate them from the pasture.

• People who assist does at kidding or collect placental or fetal waste for disposal or diagnostic evaluations should be aware of the danger of infection and are advised to wear plastic gloves. The gloves should be burned to prevent environment contamination.

• Quaternary ammonium compounds are satisfactory disinfectants.

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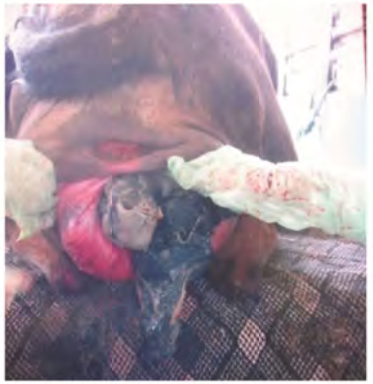
APPENDIX:Tables and Figures

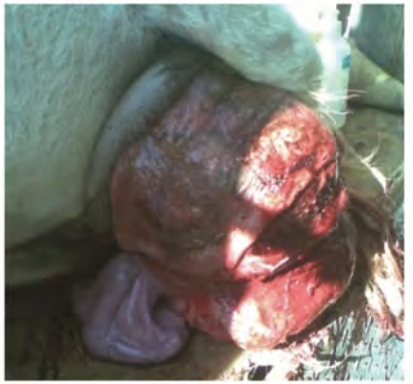
Table 13: Common diseases during pregnancy in farm animals

Female camels	Mares	Cows	Buffalo-cows	Ewes and Does
Uterine torsion, Vaginal prolapse, Abortion, Fetal emphysema	Abortion, Rupture of the prepubic tendon	Uterine torsion, Vaginal prolapse, Abortion, Fetal maceration, Fetal mummification, Hydropsy of the fetal membrane.	Uterine torsion, Vaginal prolapse, Abortion	Pregnant toxemia, Vaginal prolapse, Abortion, Fetal mummification, Fetal maceration, Abdominal hernia

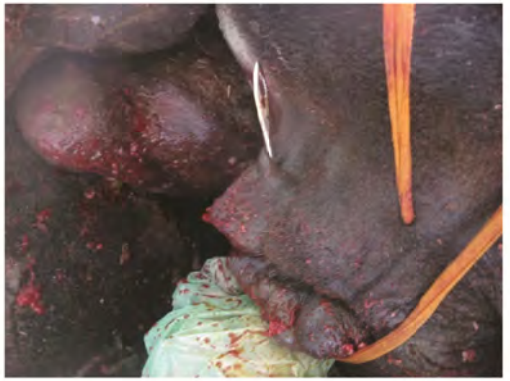
A) Severe degree of vaginal and rectal prolapse in nonpregnant female camel.

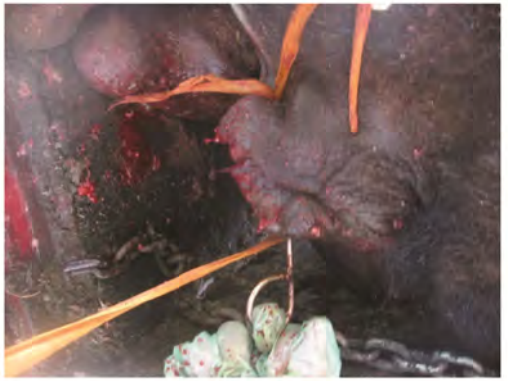


B) Complication of vaginal prolapse: passing the fetal head through the prolapsed vagina.



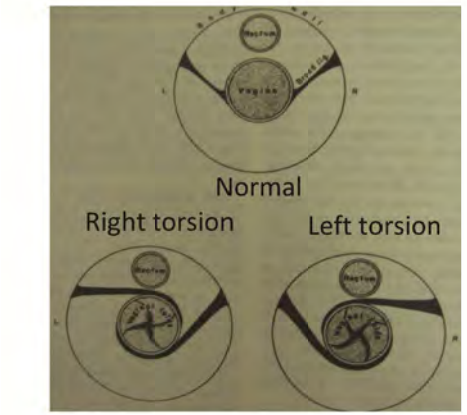
C) Complication of the vaginal prolapse: rupture of the prolapsed vagina and prolapse of the intestine.





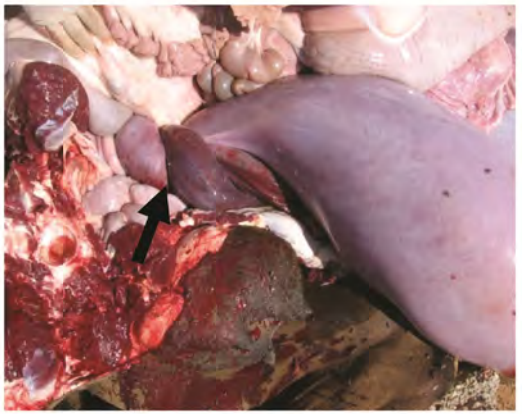
D) Treatment of complete vagina prolapse in a female camel: gentle replacement of the prolapsed part into the vaginal cavity, then suturing the vulva lips using gauze and Gerlach needle.

Fig. 45: Vaginal prolapse in female camels (*Qassim-KSA, 2007-2013*).



A) Normal and abnormal topography of the uterus

B) Site of uterine twisting (arrow)



C) Uterine torsion in a female camel (arrow)

D) Position of the fetus inside the twisted uterus

E) Slow rolling technique for correction of uterine torsion in camels

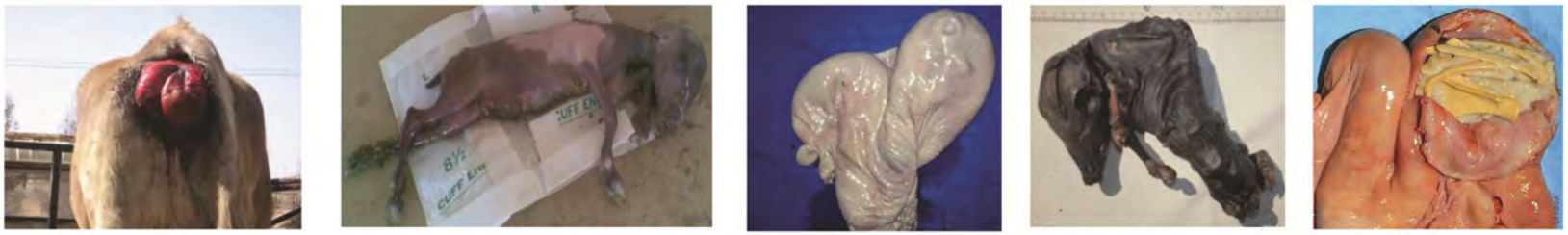
Fig. 46: Uterine torsion in female camels (*Roberts, 1986;* Qassim-*KSA, 2007-2013*).

A) Abortion in mare – 6m

B) Rupture of the prepubic tendon in a mare – advanced pregnancy

C) Rupture of prepubic tendon in a Jennet – advanced pregnancy

Fig. 47: Abortion and rupture of the prepubic tendon in mares and Jennet (*Arthur, 1964; Assiut-Egypt, 2006; Qassim-KSA, 2011*).



A) Vaginal prolapse in a cow

B) Abortion in cattle – 5m

C) Fetal mummfication in cattle

D) Fetal maceration in cattle

Fig. 48: Vaginal prolapse, abortion, fetal mummification and maceration in cows (*Assiut-Egypt 2004; Qassim-KSA, 2008*).

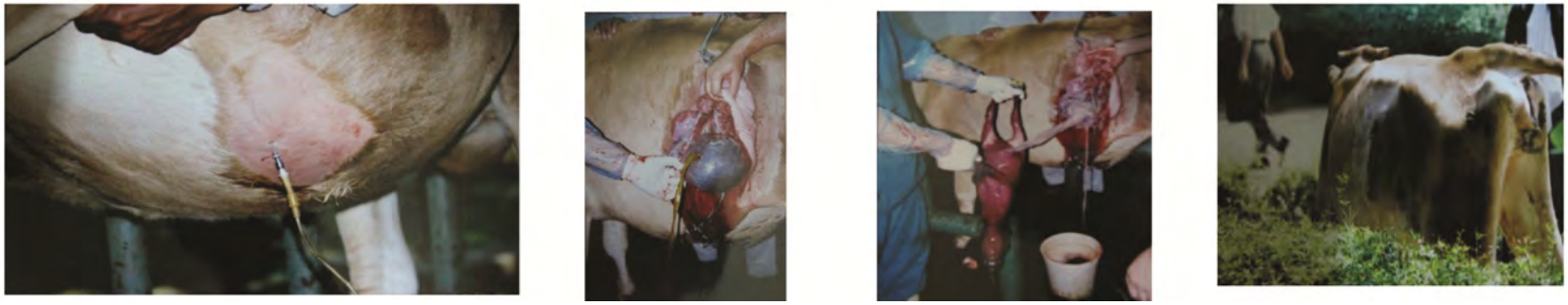


Fig. 49: Treatment of hydropsy of the fetal membrane (Hydr-allantoid) in a heifer (*Assiut-Egypt, 2003*).

A) Rapid rolling technique

B) Slow rolling technique using a plank

Fig. 50: Correction of uterine torsion in cows (*Arthur, 1964; Berlin-Germany, 1999*).

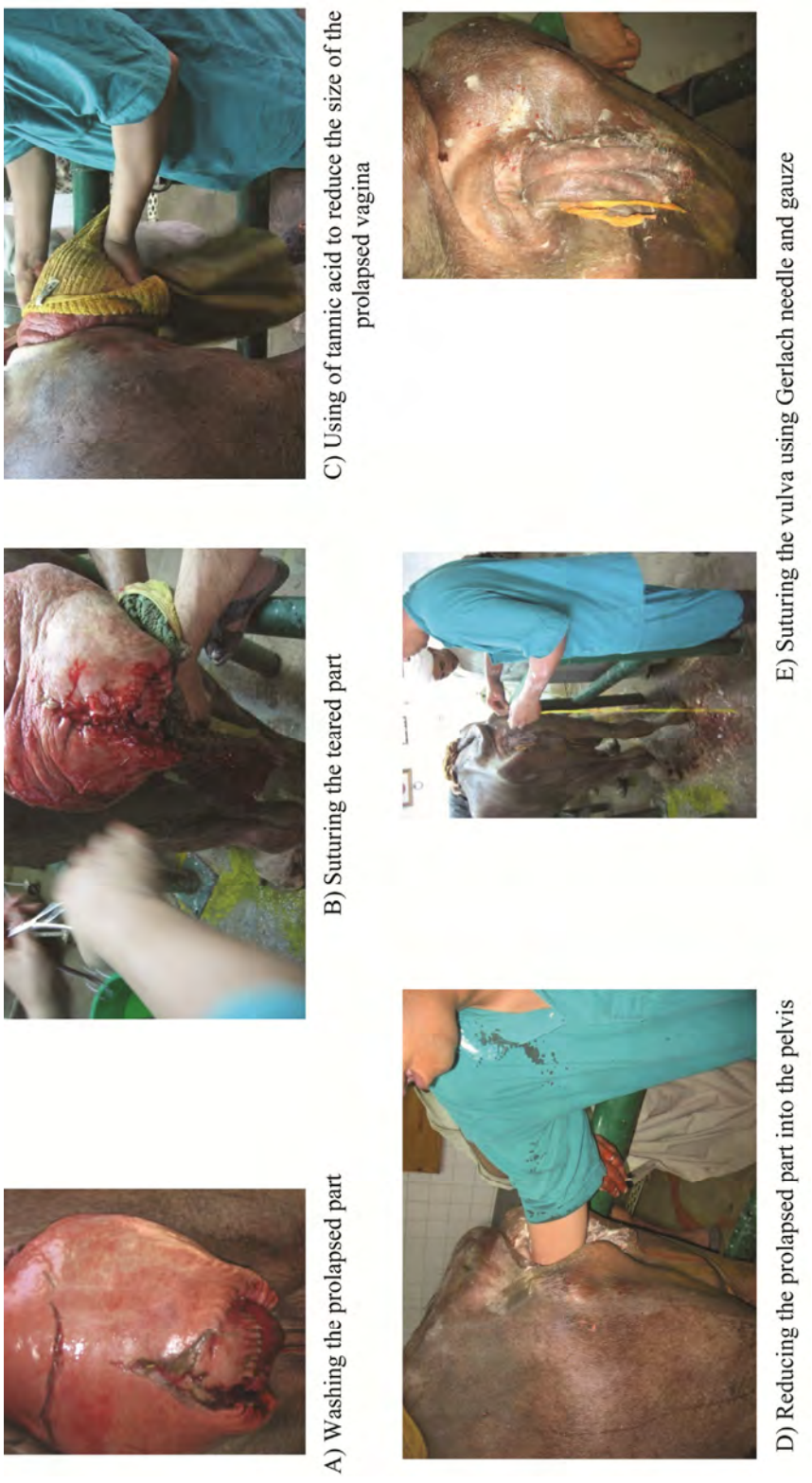


Fig. 51: Treatment of complete vaginal prolapse in a buffalo-cow (*Assiut-Egypt, 2012*).

Fig. 52: Diseases during pregnancy in ewes and does (*Qassim-KSA, 2007-2013*).

Chapter 8

Parturition

Parturition is the act or process of giving birth to offspring. The terms used to describe parturition vary with the species of animal it is being used to describe. The following are examples of parturition terminology: A cow, a buffalo cow, and a female camel *calve*s and give birth to a *calf; a* ewe *lamb*s and give birth to *lambs; a doe kids and* give birth to *kids; and a* horse *foals* and give birth to a *foal.* A number of physical, physiological and hormonal changes take place to prepare the dam and fetus for parturition. During most of the gestation the fetus is lying on its back with feet pointing up within the uterus. In the last month of gestation, the fetus will r*otate into birth position* in the ewe, mare and cow. Physiologic changes that take place in the last days of gestation are: 1) *Expansion of the pelvis* - This takes place as a result of secretion of the hormones *Relaxin* and *Estrogen* and result in enlargement of the birth canal in preparation for expulsion of the fetus. The pubic symphysis actually demineralizes, allowing more expansion of the birth canal as necessary during parturition. 2) *Sinking around tailhead* - The soft tissues around the tailhead appear sunken and the tailhead is more prominent in the last day or so prior to parturition. This is due to relaxation of the pelvic ligaments under the influence of the secreted hormones. The onset of parturition is thought to be triggered by release of cortisol by the fetus into the maternal circulation. This results in increased production and release of estrogen by the placenta. Estrogen causes the muscular wall of the uterus (myometrium) to begin contracting and preparing to expel the fetus. The uterus releases prostaglandins (PGF2α), which, in turn, causes regression of the corpus luteum and a drop in progesterone levels. Since progesterone inhibits contraction of the uterine muscles, the drop in progesterone further stimulates uterine muscular contractions.

As parturitions approaches, a number of additional physical and physiological changes may be observed: 1) *Vulva softens and becomes swollen; 2) Cervix becomes dilated*; 3) *Mucus stringing from the vulva*. This is indicative of cervical dilation and expulsion of the mucus plug which sealed off the uterus to protect it from invasion of microorganisms throughout pregnancy. 4) *Change in body temperature* of dam. The body temperature drops. 5) *Rupture of the amniotic sac*. This results in expulsion of amniotic fluid. 6) *Dripping milk* from the teats.

In addition to the physiological signs of impending parturition, a number of behavioral signs can be observed, including: 1) *Isolation* from the rest of the flock or herd. 2) *Off food* - most animals will stop eating the day of parturition. Horses are the exception to this and many will continue to nibble food, even during parturition. 3) *Distress, discomfort* - this can be evidenced by restlessness,

circling, pawing, biting or kicking at the flank, crying, bawling and groaning. 4) *Sweating* - horses in particular may sweat across the shoulders and flanks.

Stages of Parturition

There are also 3 stages of parturition. It is important to recognize each stage and monitor the dam to make sure she is progressing from one stage to the next in a timely fashion. If parturition is not progressing in a timely fashion, it may be necessary to assist the dam in the birthing process. In extreme cases, it may be necessary to call on the assistance of a veterinarian.

The **First Stage of Parturition** is the preparatory stage. During this stage, a number of important things happen, including: 1) Positioning of the fetus for birth - The normal position of a fetus is with the front feet pointing out the cervix, right-side up with the chin resting on forelegs. With cattle, sheep and horses, any other position is considered an abnormal position and may result in dystocia. 2) Dilation of cervix. 3) Exposure of fetal membranes through the vulva with possible rupture.

The **Second Stage of Parturition** is the expulsion stage. During this stage, the following takes place: 1) Uterine contractions intensify, leading to abdominal pressing by the dam, followed by and expulsion of the fetus. In the cow, this stage of parturition can take up to 4 hours; in the mare, the foal must be delivered within 15 minutes of the rupture of the amniotic sac, or the foal is likely to suffocate. The ewe usually delivers each lamb at about 15 minute intervals. The sow normally delivers piglets at approximately 5-6 minutes intervals. One should assist the dam only after the cervix is fully dilated or you may damage the cervix and uterus.

The **Third Stage of Parturition** "Cleaning Stage": During this stage, the placental membranes (afterbirth) are expelled. This happens after each birth in cattle, sheep and horses. It is very important to make sure that the animal finishes the third stage of parturition, because any remnants of placental membranes left in the uterus will serve as a nidus for infection and could lead to the death of the dam. In the horse, the placental membranes should be expelled within 15 minutes of expulsion of the fetus. After the appropriate length of time, if the membranes are not expelled, the animal has a retained placenta.

The signs of approaching parturition, parturition process, and methods of induction of parturition are shown in Tables (14-15) and (figs 53-56).

8.1. Parturition in Female Camels

8.1.1. Endocrinology of parturition

It appears that expulsion of the fetus in the camel is preceded by the attainment of a minimum level of plasma progesterone and high levels of estrogen. The average concentration of P4 recorded in periparturient female camels was around 4.0-4.5 ng/ml at 55-31 hrs prior to parturition and declined

slightly to measure 3 ng/ml at parturition. A further decline in P4 concentration to 1.6 ng/ml occurred after expulsion of the fetus. The average concentration of E2 increases slowly and steadily after the 39th week and measured more than 50, 100, 250, 300 and 375 pg/ml at the 42nd, 45th, 47th, 49th and 52nd weeks of gestation, respectively. It declined in periparturient females to 92.2-243 pg/ml at 1-55 hrs before calving. It further decline sharply to 23.3, 5.6 and 6.6 pg/ml at 5, 11 and 17 hrs after calving.

8.1.2. Parturition process

The premonitory signs of approaching parturition include segregation from the herd, restlessness, increasing humming, and relaxation of the sacroisciatic. However, the presence of colostrum in the udder remains the best sign of approaching parturition in the camel, as in the cow and mare. The average time for the complete process of parturition is 370 min. The body weights at birth of the newborn males and females are 31and 24.5 kg, respectively. Standing and first suckling by the neonatal camels occur at 68.6 and 98.6 min, respectively. There is no specific time for labour and camels calve throughout the day. Calf mortality is high during parturition.

8.2. Parturition in Mares

8.2.1. Endocrinology of parturition

Parturition is rapid in the mare, and its endocrine control is poorly understood. It is clear, however, that the hormonal changes are dissimilar to those observed in many other domestic animal species.

Progesterone: In contrast with ruminants, in which there is a gradual decline over several days, plasma progestin concentrations in mares decrease only 48 hours before parturition. However, progesterone can be detected only in minimal amounts in plasma of late pregnant mares, and progestins in the maternal circulation consist mainly of 5α-pregnanes. On the assumption that these substances are biologically active in the horse, the pre-partum changes in progestin concentrations could be important factor in the regulation of oxytocin release and the timing of parturition. From day 200, all CLs have degenerated and progesterone is produced principally by the feto-placental unit. Progesterone concentrations are low in the third trimester of pregnancy. Progesterone concentrations show a rapid rise in the last 30 days of pregnancy. Progesterone concentrations peak 2–3 days before parturition and decline to reach basal values after parturition. These changes are unlike those of other domestic species.

Estrogen: High concentrations of estrogen are produced by the feto-placental unit from day 200 onwards. Estrogen concentrations do not increase further prior to parturition. Rather, estrogen concentrations start to decline from day 300 onwards. The mare therefore undergoes a change in the progesterone: estrogen ratio.

Prolactin: Prolactin is secreted from the anterior pituitary gland and appears to be regulated by dopamine. Increased secretion of prolactin occurs in the last few days of pregnancy. This may be associated with changing concentrations of oestrogen and progesterone. Prolactin is necessary for the completion of mammary development and the initiation of milk secretion. Concentrations remain high after parturition.

Relaxin: Relaxin is produced primarily by the placenta and serves to cause relaxation of the pelvic ligaments, pubic symphysis and components of the caudal reproductive organs. Concentrations increase gradually from approximately day 250 of pregnancy. Increased concentrations cause softening and increased distensibility of target tissues. Concentrations peak during second stage parturition.

Prostaglandin: Prostaglandin is produced by the feto-placental unit. Increased synthesis of prostaglandin is probably stimulated by the increased oestrogen concentrations of late pregnancy. A gradual increase in the prostaglandin metabolite occurs in the last few months prior to parturition. A significant increase is present in the last two weeks of pregnancy. Concentrations peak at the end of first-stage parturition.

Oxytocin: Oxytocin is synthesized in hypothalamic neurons and is transported along their axons to the posterior pituitary. It is released in response to tactile stimulation of the reproductive organs. Concentrations increase prior to second-stage parturition. A large surge of oxytocin is probably the stimulus for the onset of second stage parturition. Oxytocin increases myometrial contractility. Oxytocin is produced in lower concentrations to aid expulsion of the placenta. After parturition stimulation of the mammary gland causes oxytocin release and a subsequent increase in intramammary pressure and milk ejection.

8.2.2. Parturition process

In the first -stage of parturition the mare is restless, looks at her flanks and shifts her weight from one hind limb to another (these signs can also be seen for months before foaling). Slackening of the sacro-sciatic ligaments and relaxation of the vulva are inconsistent signs. The escape of a honey-like precursor of colostrum (wax) onto the ends of the teats is a good sign that the mare is in the first stage – but some mares expel obvious milk for days before foaling and others never wax-up. The intensity of the signs depends on the mare and her environment. Mares in first-stage parturition may hold their breath and grunt, but they do not normally strain; straining is explosive and expulsive and occurs during second stage. The cervix may be dilated and feet may be found presented in the vagina before the onset of the second stage.

The second stage of parturition commences with the onset of abdominal contractions. The cervix opens relatively quickly to allow the separating chorioallantoic membranes (CAM) to bulge into the vagina. Eventually, the pressure in the vagina causes either the CAM to rupture, with the visible loss of allantoic fluid from the vulva, or the mare to strain. Either event marks the beginning of second-stage parturition and has evoked Ferguson's reflex, i.e. vaginal distension causes oxytocin release and further myometrial contractions; if the CAM hasn't ruptured, it does so now. The mare is

usually, but not always, in lateral recumbence. Straining involves tensing of abdominal muscles and rigidity of all four limbs. The amnion, a glistening white membrane, soon becomes visible at the vulva containing fluid and/or a fetal foot. Both front feet (one further forward than the other) and the nose should appear in quick succession. After expulsion of the head the mare may stand and even eat, or may roll to change the position of the foal. Further straining ensures delivery of the chest and hips. If left undisturbed, the mare may lie for some time with the foal's hind limbs in her vagina. The foal is born in the amnion, but ruptures this when it attempts to sit up. Movement of the dam or foal causes the umbilical cord to rupture close to the abdominal wall. The mare's instinct is to lick the foal dry, but not to eat the membranes. Second-stage parturition usually occurs at night (mostly from 6 pm to 6 am), and lasts 5–25 (mean 15) minutes.

The third stage of parturition "Expulsion of the fetal membranes" usually occurs within three hours. The mare may show signs of abdominal pain due to continued uterine contractions. The weight of the amnion gently pulling on the CAM via the umbilical cord causes separation from the uterine wall. The CAM is turned inside out during expulsion. The membranes should be kept and inspected to ensure that they are complete.

Induction of parturition

The main prerequisite is to determine whether the fetus is capable of surviving extra-uterine life. Several physiological processes ensure that the fetus will survive after birth. The normal fetus must have appropriate energy reserves, functional lungs and gut, and the ability to suck, swallow and maintain body temperature after delivery. Several parameters have been used to indicate fetal and maternal 'readiness for birth': 1) *Adequate gestational length* – at least 330 days; 2) *adequate mammary development and milk/colostrum production*; 3) *Suitable softening of the cervix*.

Methods of parturition induction:

1) Low-dose oxytocin regimes (10 IU intravenously) per mare Treatment is repeated every 20 minutes until parturition commences. Most mares respond within 15–90 minutes. 2) *High-dose oxytocin regimes.* (40 IU intramuscularly; or 60–120 IU is diluted in 1 l of saline and the mares infused intravenously at a rate of 1 unit/minute). These regimes appear to produce a longer parturition than the lower-dose regimes. 3) *Twice the luteolytic dose of prostaglandin:* Most mares undergo parturition within four hours. The interval to parturition may however be up to 56 hours. Parturition may take longer than in spontaneously-foaling mares or those induced with oxytocin. Oxytocin regimes are probably the methods of choice but currently there is little evidence to demonstrate differences in neonatal survival with any regime.

8.4. Parturition in Cows

8.4.1. Endocrinology of parturition

In one study, the gradual pre-partum rise in fetal plasma cortisol during the last week of gestation (from 10–20 ng/ml 7 days before parturition to 51 ± 5 ng/ml in the last 3 h before delivery) was much less marked than the abrupt increase immediately after birth when the cortisol concentration invariably doubled. Maternal plasma estrogen increased from 0.35 ± 0.04 ng/ml to 1.20 ± 0.11 ng/ml during the week before parturition. Progesterone concentrations remained stable until a sudden fall 1–2 days before delivery. The slight alterations in maternal plasma cortisol during this period were not statistically significant. The maternal plasma estrogen levels were higher in the uterine vein than in the periphery, whereas uterine venous progesterone concentrations were significantly lower than in the peripheral circulation.

8.4.2. Parturition process

The **first stage** is characterized by initiation of myometrial contractions and ends by complete cervical dilation - usually 2 to 6 h in duration. Uterine contractions push the fetus towards the cervix. Pressure by fetal fluids from the 1st water bag (chorioallantois) assists in dilation of the cervix. Interval between rupture of the 1st water bag and the 2nd water bag (amnion) averages about 1 h; “slimy fluid” from the amnion helps with lubrication. Complete dilation occurs when the presenting part of the fetus enters and exerts pressure on the cervix; therefore oxytocin is released (Ferguson’s reflex) from the posterior pituitary causing an increase in myometrial contractions

The **second stage** is characterized by expulsion of the fetus. It usually lasts 0.5 to 1 h in duration. Distention of the cervix and vagina by the fetus increases abdominal straining; therefore oxytocin is released from the posterior pituitary causing an increase in myometrial contractions. Once the head is past the vulva, usually the rest of the body follows easily.

The **third stage** is characterized by expulsion of the fetal membranes - usually occurs within 12 h of calving. Separation of the fetal cotyledons from the maternal caruncles is due to: structural changes in the placenta following rupture of the umbilicus and decreased blood flow leads to a collapse of the placentome.

Induction of parturition

The use of exogenous estrogens with dexamethasone for the induction of parturition in cattle had no significant effect on the time interval from injection to calving, calving difficulty, mothering ability or calf vigor when compared to inductions using dexamethasone alone.

Two experiments were designed to test the hypothesis that induction of parturition in the cow would be more predictable with the simultaneous use of a combination of cloprostenol and dexamethasone than with either hormone used alone. In experiment I, all 19 beef cows treated with 500 μg cloprostenol and 25 mg dexamethasone in combination calved within 72 hours whereas dexamethasone (n = 19) or cloprostenol (n = 16) treatments alone each resulted in two

induction failures. In those cows successfully induced, the mean interval from treatment to birth was 34.6 ± 1.4 hours for the cloprostenol plus dexamethasone group, 43.3 ± 2.4 hours for the dexamethasone group and 44.9 ± 2.1 hours for the cloprostenol group. Control cows (n = 15) did not calve during the first 72 hours after treatment with saline. The incidence of retained placenta ranged from 19 to 53% in induced groups whereas placentae were not retained by cows in the control group. In experiment II, all 30 beef cows in the cloprostenol plus dexamethasone group calved within the 72 hour limit, with a mean interval of 39.1 ± 1.0 hours. Twenty-six out of 31 cows calved within 72 hours with a mean interval of 51.9 ± 3.4 hours after a single injection of cloprostenol and 29 of 33 cows calved within 72 hours with a mean interval of 52.6 ± 3.3 hours after two injections of cloprostenol, 12 hours apart. Five of 34 control cows calved within 72 hours of time of treatment. The incidence of retained placenta was again high in induced cows. Results indicate that the simultaneous administration of cloprostenol and dexamethasone does constitute a safe, reliable and effective method of inducing parturition in the cow.

8.5. Parturition in Buffalo-Cows

8.5.1. Endocrinology of parturition

The concentration of plasma progesterone, oestradiol-17 beta, estrone sulphate, corticosteroids and 13,14-dihydro-15-ketoprostaglandin F2α (PGFM) was measured in 12 buffalo cows during the whole period of gestation, around parturition and for 15 d postpartum. The concentration of progesterone and estradiol-17 beta increased slightly during the first 2 months (3.5 ng/ml) and 4 months (14.8 pg/ml) of pregnancy, respectively. Their values remained consistently below these levels until near the end of the pregnancy period when progesterone concentrations decreased at d 7 prepartum (0.9 0.1 ng/ml) and estradiol-17 beta increased markedly at d 10 prepartum (26.3 pg/ml). Progesterone showed basal values (< 0.5 ng/ml) from d 4 prepartum to d 15 postpartum. Oestradiol-17 beta concentrations were maximal (82.8 pg/ml) during labour and returned to their basal values (< 12 pg/ml) at d 5 postpartum. The concentrations of estrone sulphate remained low (< 140 pg/ml) during the first half of gestation period. It increased sharply thereafter to 5620 ± 116.5 pg/ml by 30 d prepartum and afterwards declined to about 50% of this value before calving reaching basal level (< 80 pg/ml) at d 2 postpartum. The concentration of corticosteroids fluctuated narrowly (1.7 ng/ml) throughout gestation, increasing significantly ($P < 0.05$) at d 12 prepartum (5.3 ±1.8 ng/ml) and peaking to 16.8 ng/ml at the moment of delivery. Its value declined below 3 ng/ml from d 3 postpartum onwards.

The external signs of approaching parturition in buffaloes are similar to those in cattle. Certain changes in the morphology and behaviour of pregnant buffalo cow approaching parturition provide sufficient ground for isolating it in a calving pen and observing calving. A day or two before calving, the cow may become restless and keep to a small isolated area, which she defends against other cows. Restlessness is the most frequently observed behavioral change when parturition is imminent in

buffalo cows, and it has been described as increased walking, increased mobility, getting up and lying down, pawing the ground, Licking flanks and switching the tail.

The percentage of time resting in semi-lateral recumbence increased and standing and ruminating decreased during the last 12 h before parturition. Also increase the frequency of vocalization is an obvious sign of approaching parturition in buffalo-cow. Enlargement of the udder begins about 30 days before calving. Studies on the calving behaviour in buffaloes have been reported by, udder and teats distension occurred 2.4 and 1.8 days before calving, respectively.

Visible pre-parturient changes of the vulva (Hyperaemia and oedematous swelling) start on average 22 days (range 7-80 days) before parturition in swamp buffaloes. Cervical discharge is only visible for 14 days pre-partum, its consistency is viscous and of a white muddy colour in the beginning, but immediately before birth, the mucus increases and becomes clear and stringy with a thread-like viscous consistency. The animal becomes restless and anxious with dilated pupils at the onset of parturition.

Between several hours and two days before calving , a loosening of the sacro-sciatic ligament is to be clearly perceived, resulting in raising the root of the tail and the appearance of flank hollow with a tense and hanging abdomen. The cow becomes slow and sluggish and the hind limbs are spread due to this relaxation of ligament. A staggering gait is observed in some buffalo cows of heavy body size and voluminous udder. The time from the first signs of calving until parturition is variable and was varied from 14h to 45 min. before birth of the calf.

8.5.2. Parturition process

The onset of labour in the standing buffalo cow is marked by extending the tail of the animal, bending of the hip joint and straddling of the hind legs. In lying position, there is also stretching of the neck and limbs. Parturition is completed in Buffalo through the following three stages:-

The first stage of calving starts with irregular, intermittent, and uncoordinated contractions of uterine muscles. When the uterine contraction becomes intense and regular, the animal attempts to push the fetus out of the uterus. The water bag appears at the vulval lips and subsequently, ruptures and the allantoic fluids flow from the vulva. The fetus and amniotic fluids flow through the cervix and vagina. This stage takes 5-255 min or 20-355 min. The first stage of labour lasts 1 to 2 hours, being longer in primiparous than in pluriparous buffaloes.

The second stage starts with intensive contractions start and continue in quick succession until the extremities of the fore limbs are pushed out. This is quickly followed by the appearance of muzzle and head of the fetus. In most cases, calving takes place in recumbence. Buffalos rise quickly after the expulsion of the fetus and become restless and anxious until they approach the calf. This stage may take 10-213 min in a normal delivery. The third stage of calving is completed with the expulsion of the placenta. This may take from 30 min to more than 8h in a normal delivery, and should not be allowed to hang for more than 18h. Fetal membranes are expelled normally 4 to 5 hours after delivery of fetus. The placenta is expelled after about 3 h 50 min. the average weight of the fetal fluid amounts to 3.5 kg. The total time required for complete delivery is highly variable and it is affected by breed, health,

intensity of labor pain, parity and the environment. Average duration of calving in Murrah and Nili – Ravi buffaloes is about 515 and 276 min, respectively. Normal calving is about 65% of buffalo occurs either in sternal or lateral recumbence, while 35% of delivery while standing. Most births take place in the small hours of the morning, but some occur at other time. All calves are born in anterior presentation, dorsal position and posture of full extension of the fore limbs.

In 80% of the observed cases of calving, the buffalo remains in the standing position during the whole parturient phase. In others, birth commences with appearance of the first body parts of the fetus while the cow is in a lying position, however, towards the end of the expulsion stage, the dam rises to a standing position where by the calf drops to the ground. The majority of buffaloes like seclusion for calving and the presence of strangers may cause serious disturbance and lead to calving problems.

8.6. Parturition in Ewes and Does

8.6.1. Endocrinology of parturition

In the ewe, maternal plasma progesterone declines 7 to 15 days before delivery while in goats such a decline is noted 24 hours before delivery. Estrogens increase during the last days in ewes and gradually in goats. These events stimulate the muscles in the uterus to contract. The fetus and placenta are expelled and this is followed by the involution (shrinking of the uterus to normal size).

In one study, blood samples were taken once a day for 3 days before labour and for 3 days afterwards and at predetermined phases during labour from 6 goats. Two of the goats delivered one kid, and four had twins; all kidded without help. The cortisol concentration peaked when the first kid were born. The adrenaline and noradrenaline concentrations increased in association with expulsion of the first kid. The α-endorphin concentration increased during labor in goats. The met-enkephalin concentration fluctuated in goats. The oxytocin reached its highest levels just as the feet of the first kid became visible, and vasopressin peaked as the head emerged. Blood pressure and heart rate during parturition rose in parallel with increases in plasma adrenaline and noradrenaline concentrations

8.5.2. Parturition process

The first stage of parturition lasts from 2 to 12 hours, the time during which the cervix dilates. During this stage, dams will try to isolate themselves. In a crowded barn, this may be in a corner or up against a wall. The dam acts uncomfortable, getting up and down, lifting her lip, pawing the ground, and frequently urinating. Ewes and does do not “push” at this stage but the uterus is contracting causing dilation of the cervix. Some dams seem to stare off into space and then go back to chewing their cud or eating. The second stage of parturition is expulsion of the baby. This stage is fairly quick, only lasting 1 to 2 hours. The water bag may be observed followed by the feet and the head. There should be steady progress once the water bag is observed or appearance of the feet. If the dam strains

longer than 45 minutes without producing a baby, she should be checked for problems. Dams may rest between delivering twins, but twins should be delivered within 45 minutes of the first delivery.

The third stage of parturition is expulsion of the placenta. The placenta should pass within 8 hours of lambing. If the placenta retains, the dam's appetite should be monitored as well as her temperature for a fever.

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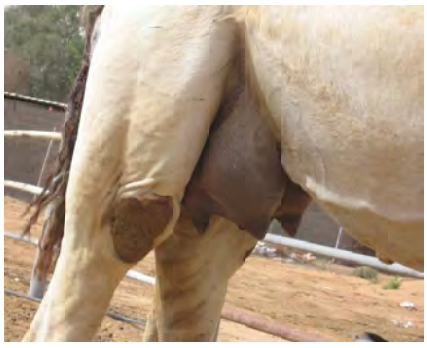
APPENDIX:Tables and Figures

Table 14: Parturition characteristics in farm animals

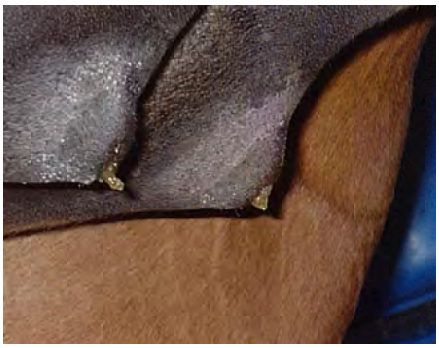
	Female camels	mares	cows	Buffalo-cows	Ewes and does
Signs of approaching parturition: Udder	edema (2w)	edema (2w)	Edema (4w)	Edema (4w)	Edema (4w)
Teats	erection, colostrums (12-48h)	waxing (12-48h)	Erection (48h)	Erection (48h)	Erection (48h)
Vulva	enlargement (1-2d)	enlargement 1-2d	Enlargement (6w)	Enlargement (2w)	Enlargement (1-2d)
Vaginal discharge	no	no	Clear, viscous	Clear, viscous	no
Pelvic ligaments	sinking (10-15d)	sinking (21d)	Sinking (10-15d)	Sinking (10-15d)	Sinking (10-15d)
Abdomen	enlargement	reduction (1w)	Enlargement	Enlargement	Enlargement
Body temperature	decrease 1-1.5 °C (12-24h)	increase (1 °C)	decrease	decrease	decrease
Parturition: First stage (opening of the cervix)	48h	1-4h	3-12h	3-12h	3-12h
Second stage (expulsion of the fetus)	5-50min	5-15min	1-3h	1-3h	0.5-2h
Third stage (expulsion of the fetal Membranes)	0.5-3h	0.5-4h	1-8h	1-8h	1-8h

Table 15: Induction of parturition in farm animals

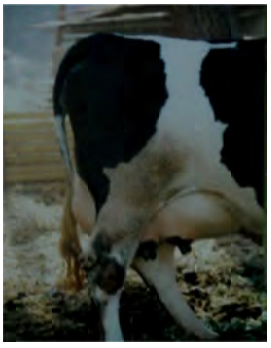
	Medicaments	Response (time to induction of parturition)
Female camels, cows, buffalo-cows	Dexamethasone (10-30 mg)	3-7 days
	Prostaglandin $F_2\alpha$ (25 mg dinoprost; 500 µg cloprostenol)	2-3 days
	Estradiol benzoate (80 mg)	4-6 days
Mares	Dexamethasone (100 mg/4days)	6-7 days
	Prostaglandin $F_2\alpha$ (10 mg dinoprost)	3 h
	Oxytocin (10 IU)	10-60 min
Ewes, does	Dexamethasone (10 mg)	2-3 days
	Prostaglandin $F_2\alpha$ (15 mg dinoprost; 250 µg cloprostenol)	2-3 days
	Estradiol benzoate (15 mg)	4-6 days



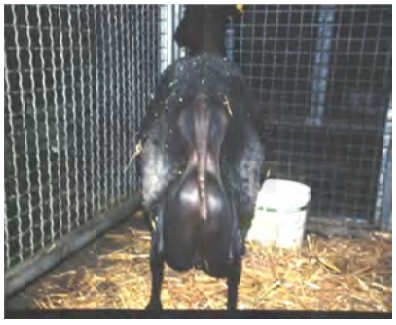
A) Edema of the udder and erection and shorting of the teats in a female camel – 48h before parturition



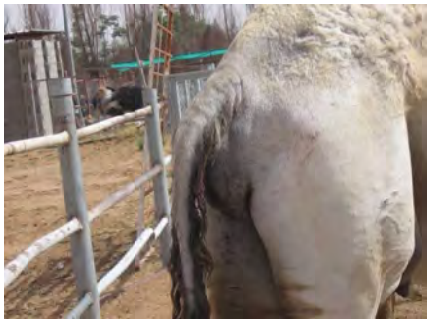
B) Ejection of colostrums from the teat orifice in a mare– 24h before parturition



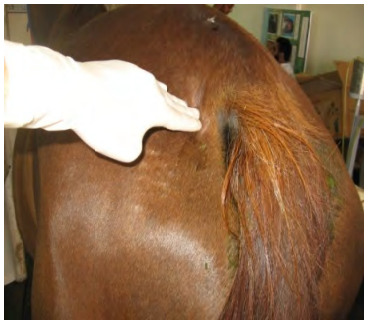
C) Edema of the udder in a pluriparous cow- just before parturition



D) Edema of the udder in a ewe – 72h before parturition



E) Edema of the vulva in a female camel – 48h before parturition



Relaxation of the pelvic ligament in mare – last stage of pregnancy

Fig. 53: Signs of approaching parturition in farm animals (*Assiut-Egypt, Berlin-Germany, Qassim-KSA, 1988-2013*).

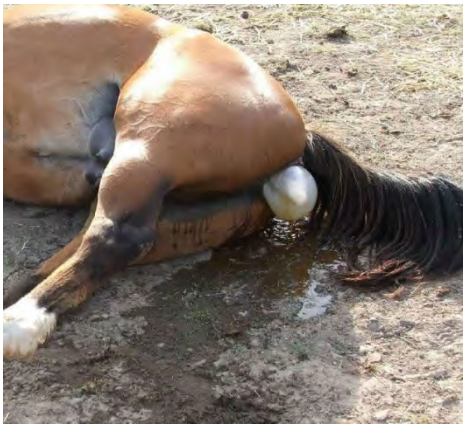
A) The second stage of birth – appearance of the head and two forelimbs

B) The mother lies down to facilitate the process of parturition

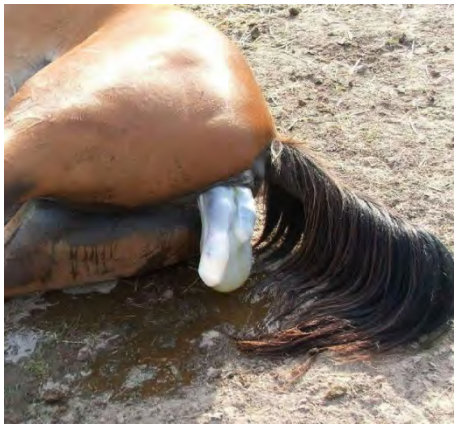
C) The mother takes a lateral recumbent position to improve the abdominal pressure

D) The mother after completing the second stage of birth – expulsion of the fetus

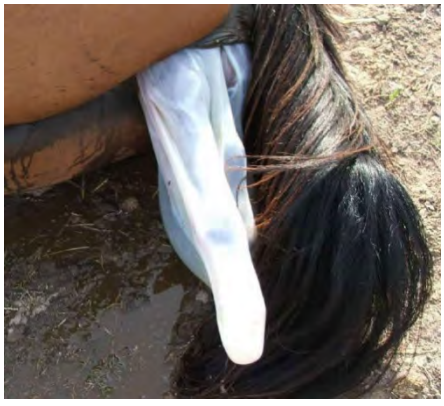
Fig. 54: Parturition process in a female camel (*www.popular.pics.com*).



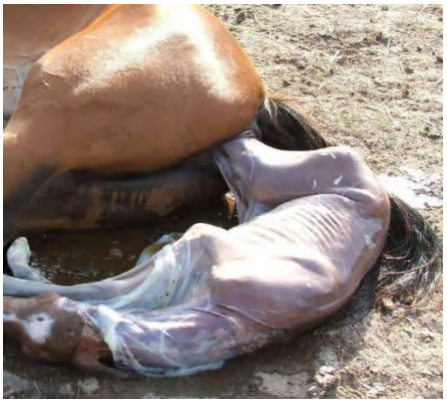
A) appearance of the forefeet



B) passage of the shoulder through the birth canal



C) The appearance of the head and two fore limbs, the fetal membranes are still intact



D) Completion of the second stage of labor

Fig. 55: Parturition process in a mare (*www.en.wikipedia.org*).

A) Starting the second stage of labor – rupture of the fetal membrane

B) The mother takes a lateral recumbent position

C) Appearance of the head and two fore limbs

D) Progressing of the second stage of labor

E) Completion of the second stage of labor

F) Licking of the fetus following delivery

Fig. 56: Parturition process in a cow (*Assiut-Egypt, 1992*).

Chapter 9

Dystocia

Dystocia (antonym eutocia; Greek: tokos "birth") is an abnormal or difficult birth or labour. Causes include maternal factors (uterine inertia, inadequate size of birth canal) and/or fetal factors (oversized fetus, abnormal orientation as the fetus enters the birth canal). Determination of the cause of dystocia requires thoroughly examination of the dam and obtains a full history of the case:

History

The first step in clinical evaluation of dystocia is to obtain as much pertinent history as possible. It can be useful to gain a brief summary of previous dystocia cases and breeder management. Information on the bull used for the mating period also may be useful.

The breeding date, if known, should be ascertained. Prolonged gestation is in most cases associated with excessive fetal size and poor viability. It is important to know how long the animal has been in labor. Dams should be allowed a reasonable amount of time to spontaneously deliver their calves.

Obstetrical examination

Confinement of the parturient dam should be in a dry, well-bedded enclosure of generous size and with sufficient illumination. A squeeze chute may be suitable for the initial examination, but the inclination of most dams to become recumbent during traction makes this choice undesirable in all but the simplest of deliveries. The clinician should observe the general condition of the patient and identify abnormalities that may potentially influence the selection of a method to relieve the dystocia or have an impact on the prognosis. Examination of the reproductive organs by palpation per rectum is indicated in only a few cases of dystocia. The most common indication for rectal palpation is to confirm uterine torsion when stenosis of the cranial vagina is detected during a vaginal examination. Pelvic deformities and exostosis may be more readily detected by palpation per rectum than by vaginal examination. Other indications for palpation per rectum include elicitation of spontaneous movements of the fetus when it is not possible by vaginal examination, confirmation of uterine rupture, and recognition of hemorrhage into the broad ligaments. Vaginal examination in dystocia almost always implies manual entry into the birth canal. Before invasion of the birth canal, the vulva and perineal area and any protruding fetal parts are washed with surgical soap and water. The hands and arms of the operator also are cleansed. It is often beneficial to pump 3 to 5 L of lubricant into the birth canal, in addition to applying lubrication to the operator’s arms. The birth canal and fetus are first examined for lesions or hemorrhage that may have been induced by previous attempts at delivery, and the

caretaker is informed of their presence. The operator then determines as accurately as possible the presentation, position, and posture of the fetus and the presence, if any, of congenital abnormalities.

In some cases, it is difficult to determine if the forelimbs or hindlimbs of the fetus are present in the birth canal; fetal elbow and fetal hock may have similar characteristics on palpation, which may confuse even the experienced operator. The limbs can be differentiated by starting at the claws/hoof and counting the joints from distal to proximal up the limb. The forelimb has a carpal joint between the fetlock joint and the elbow, whereas on the hindlimb, the hock joint is palpable immediately proximal to the fetlock joint. The ears, eyes, and mandible can be used to identify the head, whereas the presence of the tail indicates a caudal presentation. After determining the disposition of the fetus, the clinician then must determine if it is alive or dead before selecting the appropriate method to complete delivery. In cranial presentation, the interdigital claw reflex can be elicited by pinching the web of tissue between the claws. A vigorous fetus responds by withdrawing the foot. A false positive result may occur if the operator mistakes movement caused by the maternal abdominal press for that of the fetus. False negative results can occur in live calves if the head and limbs are wedged in the birth canal. The swallowing reflex is elicited by applying pressure at the base of the tongue, to which a normal calf responds by swallowing or sucking. Slow or exaggerated reactions may be associated with hypoxia or may be agonal. Slight pressure on the eyeball elicits movement of the eyeball or eyelid. The eye reflex is preserved even in severely acidotic calves. The reflexes disappear in a peripheral to central progression as the condition of the fetus deteriorates. In caudal presentation, the interdigital claw reflex of the hindlimb is similar to that in the forelimbs; however, it becomes negative earlier during the course of delivery than does the reflex in the forelimbs. Thus, the interdigital claw reflex may be negative in a viable calf, and elicitation of this reflex is a good prognostic sign. The anal reflex sometimes is used to assess the status of a fetus in caudal presentation. This reflex is elicited by pushing the examiner's finger against or into the anus. Evaluation of the response is subjective, however, and the reflex can be absent in a viable fetus. If the fetal reflexes are ambiguous or absent, the obstetrician should examine the fetal heartbeat or umbilical cord pulse. On completion of the examination and assessment of the condition of the fetus, the dam, and the birth canal, the clinician must then formulate a plan for resolution of the dystocia. The available options are mutation of abnormal presentation, position, or posture; forced extraction; fetotomy; and cesarean section. Euthanasia may be indicated in situations in which the value of the animal is limited and the prognosis poor.

The obstetrical operations

It may be prefer firstly to define some obstetrical terms:

Presentation is the relation of the spinal axis of the fetus to that of the dam. Presentation can be either longitudinal or transverse. The fetus' orientation is either **cranial** or **caudal** in the longitudinal presentation and **dorsal** or **ventral** in the transverse presentation. **Cranial longitudinal** is considered the normal presentation. Note the general trend away from the traditional use of the human descriptors of anterior and posterior in veterinary science.

Position is the relation of the dorsum of the fetus to the quadrants of the maternal pelvis. These quadrants are the sacrum, right ilium, pubis, and left ilium. Dorsosacral is considered the normal position.

Posture is the relation of the fetal extremities (head, neck, or limbs) to its own body. Extremities may be flexed, extended, or retained (usually referring to the head). Retention can be to the right, to the left, or above or below the fetus.

Mutation is defined as the process by which a fetus is restored to normal presentation, position, and posture by repulsion, rotation, version, or extension of extremities. Abnormalities of fetal posture generally are easier to correct when the dam is standing. In specific circumstances, however, placement of the dam in lateral recumbence can be advantageous, particularly if facilities such as a hydraulic tilt table or even a sloped loading ramp are accessible. For example, with retention of the fetal head, if the cow can be placed in lateral recumbence with the fetal head uppermost and the forequarters of the cow elevated slightly, mutation of the head to a normal posture can be facilitated. If mutation cannot be completed in 15 to 30 minutes, an alternate method for delivery should be selected.

Repulsion of the fetus out of the maternal pelvis into the uterine cavity where more space is available for correction is the first step in mutation. The fetus and birth canal must be well lubricated, and 3 to 5L of a water-based lubricant can be gently introduced around the fetus through a stomach tube by means of a pump. It may be necessary to abolish abdominal straining with an epidural anesthetic, but the expulsive efforts of the dam will not subsequently be available for delivery of the fetus. In general, however, use of the operator's hands and arms is recommended to reduce the risk of uterine rupture below that associated with introduction of metal instruments into the uterus.

Rotation is defined as turning the fetus on its longitudinal axis to bring it from dorsoilial or dorsopubic position to dorsosacral position. Partial rotation also is an essential component of the routine vaginal delivery technique to ensure that the fetal hips enter the maternal pelvis on a diagonal. In many cases, rotation can be accomplished by the hand and arm of the operator. By grasping the humerus of the ventral limb near the shoulder joint, the operator lifts the fetus upward and medially. If an assistant is available, traction on the dorsal fetal limb in a downward and medial direction can be applied to aid in rotating the fetus. Alternatively, the fetal limbs can be crossed and rotational force applied to bring the fetus to dorsosacral position. In difficult cases, use of a detorsion rod may be necessary, but excessive force that may result in injury to the dam and the fetus should be avoided.

Version is defined as turning the fetus on its transverse axis into cranial or caudal presentation. Transverse presentation fortunately is rare in cattle but must be converted to longitudinal presentation before delivery is attempted. Extractive force is applied to the portion of the fetus closest to the maternal pelvis while the opposite pole of the fetus is repelled. Version usually is limited to 90 degrees, and attempts to convert caudal presentation to cranial presentation are not likely to be successful and will commonly result in uterine tears.

Traction

Traction force should be applied only simultaneously with the dam's abdominal press, and tension is released when the dam ceases to strain. Obstetrical chains commonly are used to apply traction to the limbs of the fetus, but snares made from nylon or soft rope are equally serviceable. The main requirements with the material used to apply traction are that it can be easily cleaned; it can be easily released from the limb when traction is stopped, and it does not cause excessive trauma to the limb of the calf. The traditional method of applying obstetric chains is to place a loop of the chain proximal to the fetlock joint of the fetus and place a half-hitch of the chain around the pastern. It is thought that this practice spreads the extractive force over a larger area and may reduce the chances of injuring the fetus. The first step in delivering a fetus in cranial presentation is to position the head and extended forelimbs within the pelvic cavity. By applying traction alternatively on both forelimbs, the strength of one person should be sufficient to pull the head into the pelvis while the dam applies an abdominal press. Traction is not placed on the head of the fetus, but the operator's hand is placed behind the head to guide it into the pelvis. If the head cannot be brought into the pelvis, a safe vaginal delivery is unlikely and an alternate method should be chosen. After both shoulder joints have passed the pelvic inlet, traction is applied simultaneously to both forelimbs in a caudal and slightly ventral direction until the head emerges from the birth canal. The fetal hips and stifle joints constitute the next obstacle to delivery of a fetus in cranial presentation.

The fetus may be rotated from dorsosacral position to dorsoilial position to permit the widest portion of the fetal hindpart to pass through the largest diameter of the maternal pelvis. To achieve this goal, rotation of the fetus must begin as soon as the head emerges from the vulva and can be initiated by having the assistants exchange chains while they continue to apply traction. In most cases it is necessary to rotate the forepart of the fetus nearly 180 degrees to obtain the 60- to 90-degree rotation of the hindpart required for proper entry of the stifle joints into the pelvis. If sufficient rotation is not obtained by this maneuver, the operator can complete the process by wrapping the hands and arms around the fetal neck and applying rotational force. After the fetus has been sufficiently rotated, the operator stimulates it to breathe by clearing mucus from the nostrils and tickling the nostrils with a straw. After a normal breathing pattern has been established, traction is continued simultaneously on both limbs in a caudal and slightly dorsal direction and in concert with the dam's abdominal press. In cases of hiplock (engagement of maternal and fetal pelvis) , traction should be suspended and the fetus, if living, should be stimulated to breathe. The extractive force of as many as three assistants may be applied in an attempt to deliver the fetus. Continuous application of traction is contraindicated because the fetus has difficulty breathing while extractive force is employed and may die of hypoxia. If efforts to deliver the fetus are not successful, the fetus can be pulled sharply around toward the dam's flank. This tactic further rotates the fetal pelvis and causes one hip to enter the pelvis ahead of the other. Should these procedures fail to result in delivery, the final option is partial fetotomy.

Delivery of a fetus in caudal presentation poses relatively more risk to the life of the fetus than does delivery in cranial presentation. With caudal presentation, the umbilical cord ruptures early, and the fetus is likely to become hypoxic and die before the head can be delivered. Therefore, delivery of fetuses in caudal presentation should be more rapid than that of fetuses in cranial presentation, and a

decision should be made early on to resort to a cesarean section if the fetus is alive and delivery is impeded. Before attempting delivery, the fetus is first rotated into dorsoilial position so that the widest portion of the fetal hindpart approaches the widest diameter of the pelvis. Rotation can be accomplished by crossing and twisting the hindlimbs. After the fetus has been rotated, two assistants apply traction simultaneously to both hindlimbs, in concert with the dam's abdominal press, in a caudal and slightly dorsal direction.

Correction of the displaced Head

The head most commonly is deviated to the left side of the fetus and lies against the thoracic wall. The malposture is corrected by grasping the orbital grooves with the thumb and middle finger and drawing the head into the maternal pelvis. A rope snare placed behind the incisor teeth may be useful in difficult cases. Traction to redirect the head can be applied with the snare by the operator or by an assistant while with the other hand the operator guides the head and protects the uterine wall from the incisor teeth by covering the fetal mouth. The head may be deviated ventrally between the forelimbs, with the mandible resting against the sternum. A hasty examination may fail to reveal the presence of the head, and the malposture may be mistaken for a case of caudal presentation. In some instances, the malposture can be corrected by repelling the fetal forehead with the thumbs while simultaneously lifting the jaw with the fingers. Correction in more severe cases requires that one or both forelimbs be repelled and flexed at the carpus, elbow, and shoulder joints. Space is then available to convert the ventral displacement of the head to lateral displacement, which is then corrected by drawing the head into the pelvis. The induced malposture of the forelimb is subsequently corrected after the head is in its proper position. Should attempts to reposition the head by these methods not be successful, the dam can be sedated, cast, and rolled to dorsal recumbence. The fetus then falls toward the maternal spine and away from the narrow ventral portion of the pelvis, allowing the head to be more easily guided into the pelvic canal.

Correction of the displacd extremities

Mutation of malposture of the fetal extremities usually requires that the fetus be repelled out of the maternal pelvis before attempts at correction. In general, correction of flexion of an extremity is accomplished by repelling the proximal end, rotating the middle portion laterally, and applying traction to the distal end. Repelling and rotating forces can be applied with the operator's hand. Traction can be applied by the operator if sufficient space is available in the birth canal to permit the introduction of both arms, or by an assistant using an obstetrical chain or snare.

Fetotomy

If delivery by traction is not possible without danger to the dam or the fetus, the veterinary obstetrician must consider the option of cesarean section or fetotomy. A two-barrel fetotome with a smooth head made from hardened steel is used. A hardened head is required to prevent the saw wire from cutting into the head during cuts parallel to or at right angles to the head. The fetotome also has a notched plate near the handle that allows fixation of an obstetrical chain under tension to maintain proper position of the instrument relative to the fetus. Threader and brush are necessary to clear the

barrels of lubricant and debris. Several types of wire saw handles are available, and the choice depends on the preference of the operator. A Krey hook with an obstetric chain or rope attached is necessary to extract fetal parts after they have been separated. A curved wire introducer facilitates passage of the saw wire around fetal parts. If a wire introducer is not available, the fetotomy wire can be attached to an obstetric chain to assist in passing the wire around the fetal hindquarters. A good grade of saw wire should be selected. Epidural anesthesia is indicated in nearly all instances to relieve pain as well as straining. The administration of antibiotics before the commencement of the procedure should be considered. A generous amount of lubricant is required during a fetotomy to protect the genital organs of the dam, as well as the hands and arms of the operator. At least one and preferably two assistants are required to perform a fetotomy.

Indications of Fetotomy

Fetotomy is given primary consideration to relieve dystocia when the fetus is dead, and cesarean section is given primary consideration when the fetus is living. Fetotomy is useful to relieve dystocia caused by fetopelvic disproportion, pathologic enlargement of the fetus (fetal gigantism), incomplete cervical dilatation, fetal malposture and malpresentation, and fetal malformations including those resulting in fetal monsters. Fetotomy is not useful when the birth canal is obstructed or reduced in size, as in uterine torsion. Either partial or complete fetotomy may be required to relieve dystocia. A complete fetotomy usually is required to deliver oversized fetuses.

Fetotome cuts

There are three fetotome cuts based on the relationships between the direction fetotome tube and the cut area: 1) transverse cut: the fetotome tube is perpendicular on the cut area, like cutting of the extended head and neck; 2) longitudinal cut: the fetotome is parallel to the cut area, like cutting of the flexed head and neck; 3) oblique cut: the fetotome tube is oblique to the cut are, like cutting of the forelimb from the shoulder joint. To make a transverse cut, both fetotome tubes should be threaded by the wire, the fetal part should be fixed and extended by rope or chain and then pass in the loop or snare from upward to downward, the head of the fetotome then now pass and fixed by the operator hand on above the cut area. To make a longitudinal cut, only one tube is threaded, while the other end of the saw is passed around cut area, then return to pass though the other fetotome tube, then the head of fetotome tube is passed cut area. The oblique cut is made likewise the transverse cut, but the head of the fetotome is healed at the shoulder of the other side or the pelvis of the other side in case of cutting of the limb or leg, respectively.

Aftercare with Fetotomy

In most cases, the dam requires less care after fetotomy than after cesarean section. On completion of a fetotomy, the uterus is routinely lavaged with warm water to which is added a small amount of a nonirritating disinfectant or salt. Approximately 4L is pumped into the uterine cavity through a stomach tube and then siphoned out. The procedure is repeated until fetal tissue and lubricant have been removed and the efflux is clear. Dystocia is a common antecedent to secondary uterine inertia, and treatment with an ecbolic agent such as oxytocin often is indicated. Systemic

antibiotics frequently are indicated, especially in cases with protracted labor. Other supportive therapy such as administration of intravenous fluids or calcium is given as required.

Cesarean Section

Principal guidlines

Although a number of procedures are available for cesarean section and these procedures vary greatly, there are common principles that guide the veterinarian in the selection of the surgical approach and conduct of the procedure. A paramount goal of cesarean section should be to limit the contamination of the peritoneal cavity with uterine contents. Peritoneal cavity contamination, particularly in cases of dead, emphysematous fetuses, greatly increases the risk of peritonitis, limits the animal's chances of survival, and limits the surviving animal's productivity.

It is important to exteriorize the uterus. This aids in limiting peritoneal cavity contamination, thereby aiding in the prevention of peritonitis. The choice of surgical procedure has a direct bearing on whether the practitioner is able to exteriorize the uterus. Large fetuses, large animals, and small stature all limit the ability of the veterinarian to exteriorize the uterus. For example, when delivering a fetus in anterior presentation, a common method to exteriorize the gravid uterus is for the surgeon to enter the peritoneal cavity with his/her hand and identifies the gravid horn of the uterus. Then, rather than attempting to lift the entire uterus, the surgeon identifies and grasps a rear limb in the middle of the shaft of the metatarsus. The surgeon's other hand is then placed over the point of the tarsus. The limb is rocked toward the incision, lifted through the incision, and the hock is wedged in the cranioventral aspect of the incision. When delivering a fetus in posterior position, the surgeon grasps the metacarpus and the carpus is wedged in the incision. After positioning the limb in the incision, the uterus is incised avoiding cotyledons, the limb is grasped directly and traction is placed on the calf. Uterine incisions should be positioned on the greater curvature of the uterus and the incision should be placed distant from either the cervix or apex of the horn. As a general rule, an incision from either the metatarsus or metacarpus to the foot is sufficiently long to permit extraction of the calf without causing uterine tears. As the calf is being extracted, the contralateral limb is identified and exteriorized, and the uterus is brought out with the calf as it is exteriorized. Attempts to lift the entire uterus out through the abdominal incision prior to incising the uterus usually fail, due to the combined weight of the fetus and uterus. Attempts to manipulate or grasp the uterus rather than the fetus will often cause uterine tears and abdominal contamination.

The uterus should be closed with an absorbable monofilament suture on a tapered needle in a continuous inverting pattern. Sutures should be placed only partial thickness, incorporating the serosa and muscular layer of the uterus. Studies have examined uterine healing when a number of different suture materials have been used. Surprisingly, large sized, plain catgut is probably the suture material of choice. Synthetic absorbable sutures persist longer in tissues and have been associated with more dramatic scarring of the uterus. Additionally, catgut appears less prone to cause uterine tears when tension is placed on the suture line. Braided suture materials seem particularly prone to cause tearing of the uterus as they are tightened. In terms of suture patterns, the use of the Utrecht pattern with all portions of suture, including starting and finishing knots buried, appears to be the best. Exposed suture

is a nidus for adhesions following intra-abdominal surgery. In cases of an emphysematous fetus or if the uterus is friable, the surgeon may decide to oversew the uterus with a 2nd layer.

Sedating the animal with xylazine prior to uterine closure is not recommended. Xylazine has a direct myotonic effect, causing uterine contractions, which make the uterus friable. These contractions may make exteriorization of the uterus and suture placement more difficult and problematic, increasing the likelihood of uterine tears during closure. During standing approaches, the need for sedation is obviated by good restraint facilities and local anesthesia. For recumbent procedures, casting, restraint, and local anesthetics should permit the surgical approach, fetal extraction, and uterine closure. In cows that struggle or remain fractitious the surgeon may choose to administer xylazine after uterine closure is completed.

Standing left paralumber approach

The standing left paralumbar celiotomy is the most commonly used approach for an uncomplicated cesarean section. In general, paralumbar approaches are often favored by practitioners because most food animal practitioners are familiar with this approach. The approach is sufficiently similar to that used for rumenotomy and either the right or left approaches to correct abomasal displacement, so that most practitioners have a high degree of comfort with this approach. The incision is made vertically in the middle of the paralumbar fossa, starting approximately 10 cm ventral to the transverse processes of the lumbar vertebrae and continuing ventrally, far enough to allow removal of the fetus. Closure of the abdominal wall is straightforward and relatively easy. Absorbable suture is used to close the abdominal musculature. The rumen aids in retaining the abdominal viscera within the peritoneal cavity. Absolute requirements for this procedure include an appropriate restraint facility and an animal capable of standing through the entire procedure. Contraindications for this procedure include an inability of the patient to stand through the procedure and large fetuses that preclude exteriorization of the uterus. Lifting a uterus and fetus to the paralumbar incision is usually difficult and occasionally impossible for some practitioners.

Standing right paralumber approach

This approach has all the indications and contraindications of the left paralumbar approach. The additional and perhaps most important difference between the left and right paralumbar approach is the difficulty in keeping viscera in the peritoneal cavity with the right paralumbar approach. Most practitioners studiously avoid this approach; however, some practitioners feel right horn pregnancies are more manageable with the right paralumbar approach. This approach is helpful when a large calf can be palpated in the right horn with its limbs directed towards the right side of the animal with hydrotic condition of the uterus. In the case of an animal with such a condition, the location of the rumen and the increased size of the uterus seem to force the uterus into the right paralumbar fossa, permitting easier removal of the fetus, limiting abdominal contamination, and permitting the surgeon to leave substantial volumes of fluid within the lumen of the uterus. This is not to suggest that cesarean section is the treatment of choice for hydrotic conditions of the uterus; however, the practitioner is occasionally presented with cows whose hydrops condition is sufficiently advanced that it seems unlikely that the cow will survive an induced parturition.

Recumbent left paralumber approach

This approach differs little from the standing left paralumbar approach. Additional assistance is nearly always needed to cast the animal, if not recumbent already, and to place the cow in right lateral recumbence. The incision is made slightly more ventral than in the standing left paralumbar celiotomy. Exteriorization of the uterus is often difficult because the gravid uterus falls away from the incision. Closure is more difficult than when the standing left paralumbar approach is used, due to increased tension on the muscle layers, but it is rarely problematic.

Recumbent right paralumbar approach

This approach is very seldom used, as it is very similar to that of recumbent left paralumbar celiotomy and has the additional complication of not having the rumen to retain the abdominal viscera.

Recumbent ventral midline

This approach is straightforward and is most commonly used on a recumbent animal. If the incision is appropriately placed, the only body wall layers incised are the skin, subcutis, and the linea alba. Additional assistance is required to cast and position the animal for this approach. The cow is typically positioned in dorsal recumbence, leaning toward the surgeon at a 45 degree angle. Both front and hind feet are tied to a gate or wall. This positioning is critical. If the cow is positioned either in exact dorsal recumbence or leaning away from the surgeon, exteriorization of the uterus becomes problematic, if not impossible. Once the peritoneal cavity has been opened, it may be necessary to pull the greater omentum cranially to expose the uterus. Exteriorizing the uterus is facilitated by untying the hind feet only and temporarily laying the hind limbs flat on the ground. After removal of the fetus and closure of the uterus, the animal is repositioned in dorsal recumbence and the linea alba is closed. Closure of the abdominal wall is often difficult. The authors typically close the linea with polyglactin 910 (#3 Vicryl) in an everting interrupted horizontal mattress pattern. Eversion of the linea permits the surgeon to oversew the linea with relative ease and safety. Other appositional suture patterns, including simple continuous, may be used. Some practitioners may choose to close the linea alba with a braided nonabsorbable suture; however, this choice will cause carcass contamination with foreign material. A surgeon's knot, 2 overhand knots on the 1st row, facilitates appositional closure of the linea alba. In cases where closure of the abdomen wall is difficult, loosening the back legs and using Bachaus towel clamps to oppose the 2 sides of the incision will help to relieve the tension prior to tying the knots. Integrity of abdominal wall closure is critical. Less than optimal closure may result in either abdominal wall herniation or, in severe cases, evisceration of the animal. The ease with which the uterus is exteriorized with this approach makes it optimal for exteriorizing the uterus, a critical issue when the surgeon is attempting to remove an emphysematous fetus. This approach is also ideally suited to 1st calf heifers of the beef breeds, because the incision is somewhat hidden and does not involve retail cuts, suggesting that this approach would be preferable, if the producer is likely to sell the animal for slaughter soon after the procedure. This approach should be used when the large udder of older beef and dairy cows precludes extending the incision sufficiently caudal to permit ready exteriorization of the uterus, and when udder edema and the increased ventral vasculature make this approach more complicated.

The common forms of dystocia in farm animals are shown in Tables (16-21). The causes of dystocia and obstetrical operations in each species are illustrated in Figs. (57-70).

9.1. Dystocia in Female Camels

In one study encountered by the author on 60 cases of dystocia in Arabian camels, maternal and fetal dystocia represented 35/60 (58.3%) and 25/60 (41.7%), respectively. Uterine torsion was the most frequent cause of all dystocia (20/60, 33.3%). Clockwise and counter-clockwise torsion were recorded in 12/20 (60%) and 8/20 (40%), respectively. Vaginal adhesion had developed in long standing cases (6/20, 30%) and diagnosis of torsion was achieved through trans-rectal palpation. Uterine torsion was post-cervical with vaginal involvement in 8/20 (40%), pre-cervical without vaginal involvement in 2/20 (10%), the rests were difficult to determine due to presence of adhesion. The degree of uterine torsion was mild, moderate, and high in 3/20 (15%), 8/20 (40%), and 9/20 (45%) of the cases, respectively. Abnormal postures was the second important cause of dystocia (16/60, 26.7%). It included head deviations (lateral: 6/16; downward: 2/16 deviations), breech presentation (3/16), double hock flexion (2/16), double fetlock flexion (2/16), and double shoulder flexion (1/16). Feto-pelvic disproportion was the third common cause of dystocia (13/60, 21.7%). It included cases with narrow pelvis (7/13) and big-sized fetus (6/13). Narrowing cervix (5/60, 8.3%) abnormal presentation (3/60, 5%: dog-sitting 2/60, and transverse dorsal 1/60), and vaginal prolapse (3/60, 5%) were also found as causes of dystocia. The fetuses were found alive, freshly dead, dead but not putrefied, or putrefied/emphysematous in frequencies of 7/60 (11.7%), 10/60 (16.7%), 9/60 (15%), and 34/60 (56.7%), respectively. Alive fetuses were found only in dams arrived in less than three days of signs appearance. In a similar study to analyze the causes of dystocia in 17 camels for which cesarean section had to be performed at the farmer's doorstep, along with the outcome of such surgeries. Maternal causes of dystocia were common (58.82%) indications for the surgery compared to the fetal causes (41.18%). The maternal causes included uterine torsion (17.64%), cervical dilation failure (11.76%), narrow birth canal (11.76%) due to pelvic fracture or dam's immaturity, uterine rupture (5.88%), uterine prolapse (5.88%) and vaginal rupture (5.88%). The fetal causes included uncorrectable fetal malpostures (29.41%), oversized fetus (5.88%) and schistosoma reflexus (5.88%) monster. Only 35.29% of the calves could be delivered alive and the calf viability depended upon the time of referral (6h-10days) after the onset of 2nd stage of labor. The proportion of male and female calves delivered was 58.82 and 41.18% respectively. With sufficient perioperative care the dam survival was high (70.58%) and only 29.41% dams died due to severe blood loss or peritonitis. The common postoperative complications were edema at the operative site, minor wound dehiscence and subsequent herniation.

Other Reports claimed that the incidence of dystocia is low in the camels; however because of the exceptionally long neck and extremities when it occurs, it is difficult to manage. The incidence reported varies from 0.4% to 4.6%. The incidence reported for llamas vary between 2% to 5%. Data from 1660 births in alpacas (in South America) recorded an incidence of only 1.6% whereas data of 234 llama births demonstrated an incidence of only 0.4%. The incidence in Bactrian camel is exceptionally low and not reported for large number of births. In most cases of dystocia, intervention

occurs during the second stage of labor after observation of a failure of normal progression of the first stage of labor. Early diagnosis of dystocia is important; however, many times the diagnosis is very late and frequently noticed only when the limbs of the fetus are protruding through the birth canal and the animal straining vigorously. At organized farms, camels are monitored frequently around the due dates for parturition, but this is not routine for the pastoralists as they keep no records. The most important sign of dystocia is the increased duration of the second stage of labor. Dystocia should be suspected if the first stage or second stage of labor exceeds 6 and 2 hours, respectively. In addition, the dam may show signs of distress frequently alternating between standing and sitting with frequent side to side rolling and excessive straining. Many dromedary females will show diarrhea and frequent vocalization in such cases. At times, females are depressed and show no signs except slight straining and a blood stained discharge.

With regard to cesarean section in the camelidae, two operative sites are commonly used for cesarean in the camelids; the paralumbar fossa and oblique ventro-lateral. A ventral midline approach is considered to be improper due to severe postoperative complications. The left paralumbar fossa is a site on which the operation is easy to perform because the animal is in sitting position and surgeons can sit parallel to the camel. The paralumbar fossa is also preferred in llamas and alpacas with the animal restrained in right lateral recumbence. Two techniques have been described for operation at this site a) incision (30 - 40 cm long) in the middle of the fossa 6 cm below the second lumbar transverse process and parallel to the last rib extending through skin, muscles and peritoneum an oblique incision in the lower flank 10 cm posterior to the last rib In the ventro lateral approach a skin incision (30 - 40 cm) is made 5 - 10 cm above and parallel to the subcutaneous abdominal vein in an oblique fashion Alternatively the incision may be just lateral to arcus cruralis and the stifle joint on the left side in dromedary camels with the animal restrained in right lateral recumbence Ventral midline laparotomy has also been suggested for cesarean section in the South American camelids but is best performed with the dam under general anesthesia. Antibiotics and anti-inflammatory drugs are suggested to be given for 5 - 7 days along with fluids. Administration of 30 - 40 IU of oxytocin may be indicated IM to stimulate uterine contractions and hasten placental expulsion. The skin sutures heal with difficulty in the camel. Also, fluid accumulation at the operative site is common. Herniation at the operative site is a common complication. It is imperative to monitor the general condition of the patient. The first 72 h are critical as peritonitis often develop during this period. The skin sutures must only be removed after complete healing which may take up to 20 days in the dromedary camel. Less frequent complications noted in the llamas and alpacas include vaginal tears, vulvar swelling, metritis, retained placenta, hyperglycemia and uterine prolapse.

Regarding obstetrical procedures, there are three major differences between camelids and ruminants: (1) the pelvic inlet is narrower; (2) the cervix and vagina are more prone to laceration and severe inflammation (often leading to adhesions); (3) risks for neonatal hypoxia and death are increased by the forceful uterine and abdominal contractions and the rapid detachment of the microcotyledonary placenta. Consequently, (1) early recognition of dystocia is paramount, (2) obstetrical decisions and manipulations should be rapid, and (3) supportive care should be provided to the dam and fetus (if alive) before and during manipulation.

9.2. Dystocia in Mares

Despite the long limbs of foals, dystocia is uncommon in the mare. The most severe forms of malpresentation are the rarest. Dystocia more often results in death of the foal because: the mare usually continues with expulsive efforts; the placenta separates rapidly during labour and, unless the foal can breathe, it soon loses its oxygen supply and dies. Continued unproductive straining by the mare may cause damage to her reproductive organs; uterine damage during dystocia can cause fatal peritonitis or haemorrhage; retained placenta, as a result of uterine inertia following dystocia; uterine prolapse may also occur.

The foal is normally born in anterior presentation, dorsal position and extended (head, neck and forelimbs) posture. Failure to observe the fluid-filled amnion (which may be visible only during contractions) at the vulva after five minutes of second-stage parturition indicates that vaginal examination is necessary and may reveal: 1) two feet and a nose, due to feto-maternal disproportion or fetal oversize, rare in mares; 2) hydrocephalus impeding passage of the enlarged head through the cervix; 3) slow relaxation of the cervix; 3) dorsal deviation of one or both feet, if not corrected this can cause recto-vaginal trauma; 4) One foot and nose, carpal and/or shoulder flexion of one forelimb; 5) Nose only, carpal and/or shoulder flexion of both forelimbs; 6) Two limbs only, head and neck flexion; 7) Nothing palpable in the vagina, this is serious and indicates: transverse presentation; 8) posterior presentation with bilateral hip flexion (breech); 9) anterior presentation with bilateral limb and head/neck flexion.

Obstetrical operations:

Tranquillizers may make early manipulation easier, but will reduce straining when this could be helpful. Epidural anaesthesia has the same advantage and disadvantage as tranquillizers – in addition, the response is slow and variable. Introduction of a stomach tube into the trachea prevents the mare from straining. General anaesthesia may be considered for final manipulative attempts before a surgical approach. Vaginal examination should be made with a washed and lubricated ungloved hand – this aids the differentiation of the vaginal wall (if the cervix is closed), allantochorion and amnion. If the allantochorion is still intact it must be ruptured using a finger (nail), guarded knife or hypodermic needle – this membrane is very tough. Repelling any part of the fetus which is in the vagina, to allow access to flexed appendages; application of ropes to the head and fetlocks; application of blunt eye hooks; the introduction of warm water or saline into the uterus where all the natural fluids have been lost; applying traction to the foal, or attached ropes, once satisfactory posture and position have been achieved. Problems encountered during manipulation are: 1) observers often expect rapid results and do not understand the difficulties involved; 2) the mare may be uncooperative; pain (due to the mare straining) and tiredness of the operator's arms make manipulation progressively more difficult; 3) drying of the mare's vagina makes her resentful of repeated re-insertion of the arm; 4) the ruptured amnion, particularly when trying to apply ropes to the head, constantly insinuates itself between hand and foal, and prevents the rope from gripping; 5) preventing a head (which appears reluctant to be born) from flopping back into the uterus can be difficult. Whilst applying traction to a foal, always consider: is the vagina adequately lubricated?; the direction of pull – once the fetal head is clear of the vulva the foal should be pulled towards the mare's hocks; the strategy of traction – try to

ensure that limbs are pulled alternately and in unison with the mare's straining efforts – retain tension on the head; could the fetus be oversized? This is rare; have the hips locked? Once the head and forelimbs are delivered the rest of the birth should be easy. If this is not so it may be because of hip lock or hind-limb flexion (dog-sitting position); the latter cannot be diagnosed. In this case repel the foal if possible and rotate it into a lateral position – a large rotation of the front of the foal probably only affects the hips to a minor degree, and then apply traction again. When the foal is born by traction, the mare is often standing. Once the thorax starts to pass through the vulva, call for assistance to support the foal to prevent trauma from falling and premature rupture of the cord. If the mare is recumbent after delivery, pull the foal's forelegs round to the mare's head to establish contact. Allow the cord to rupture spontaneously, do not ligate it; if haemorrhage occurs apply a haemostat temporarily. After any delivery, particularly if it is easy, check for a second fetus. The time at which manipulation and/or traction will have been considered to fail will depend on many factors, not least the possibility of quick surgical intervention.

9.3. Dystocia in Cows

Causes of dystocia include a lack of expulsive force and abnormalities of the birth canal. Primary uterine inertia is characterized by failure of the myometrium to contract normally and bring the fetus into the cervical canal. The condition is encountered occasionally in cows, and causes that have been suggested include overstretching of the uterus by multiple or abnormal fetuses, a defect in the myometrium that renders it unable to contract normally, a defect in the hormonal milieu, and periparturient hypocalcemia. The dam may exhibit a few weak abdominal contractions but does not progress to the second stage of labor. On examination, the cervix is found to be dilated but the fetus has not yet entered the birth canal. The fetal membranes may be intact if labor has not been prolonged. Calves usually are delivered by gentle traction after correction of any defects in posture or position.

Secondary uterine inertia is a result of exhaustion of the myometrium after prolonged unsuccessful attempts to deliver a fetus. Treatment is directed at removing the impediment and delivering the fetus by a method appropriate for the clinical circumstances. Sequelae of secondary uterine inertia include retained placenta, delayed uterine involution, and uterine prolapse.

Delivery may be inhibited by inadequate size of the maternal pelvis, pelvic deformities or exostosis, incomplete dilatation of the cervix, vaginal cystocele, neoplasms of the vulva and vagina, remnants of the müllerian ducts persisting as bands of tissue from the dorsal to ventral walls of the vagina immediately caudal to the cervix, and uterine torsion. Stenosis of the vulva and vestibule may be the result of immaturity or may be a heritable defect in some breeds.

The fetal origins of dystocia in cattle can be divided into those caused by abnormalities of the fetus (defects in fetal disposition and various forms of maldevelopment resulting in fetal monsters) and those caused by excessive fetal size relative to the maternal pelvis (fetopelvic disproportion).

The most common cause of dystocia in cattle is fetopelvic disproportion. The situation is most common in heifers where the fetus is of normal size for its breed but the maternal pelvis is of

insufficient size (relative oversize) or the fetus may be unusually large and cannot be delivered through a pelvic canal of normal size.

A variety of malformations resulting in specific fetal phenotypes and conjoined twins have been described as sporadic causes of dystocia in cattle. Among the fetal monsters more likely to be encountered in cattle are schistosoma reflexus and perosomus elumbus. **Schistosoma reflexus** is characterized by extreme ventral curvature of the spine, so that the head is positioned near the sacrum. The abdominal and thoracic walls are not closed, and the viscera are exposed. Limbs of the affected fetus frequently are rigid because of ankylosis of the joints. **Perosomus elumbus** is characterized by a nearly normal fetal forepart but flexure and ankylosis of the hindlimbs. Vertebrae are absent caudal to the thorax, and the pelvis is deformed and flattened.

9.4. Dystocia in Buffalo-Cows

In buffaloes, maternal causes of dystocia were common (64.2%) than fetal causes (35.8%). Uterine torsion is the most frequent cause of dystocia in buffalo, followed by incomplete dilatation of cervix and uterine inertia. It is observed commonly in pluriparous animals at the time of parturition or during the last month of gestation. The condition accounts for about 30 to 50% of dystocia cases in this species. In a study carried by the author and his co-workers on Egyptian buffalo cows affected with uterine torsion , the main clinical signs of torsion included straining or colic for prolonged time, reduction in feed intake and constipation in 88/126 (69.8%), 72/126 (57.1%) and 13/126 (10.3%) of the cases, respectively. The mean duration of torsion (from appearance of the clinical signs until treatment) was 68.7 ± 10.6 h (range: from 20 to 168 h). The torsion occurred during pregnancy and at full term in 74/126 (58.4%) and 52/126 (41.6%) of the cases, respectively. Torsion was post-cervical with vaginal involvement in 124/126 (98.4%) of the cases. The rest were pre-cervical without vaginal involvement. The degrees of uterine torsion were mild, moderate, and high in 21/126 (16.7%), 39/126 (31%), and 66/126 (51.3%) of the cases, respectively. Clockwise torsion was present in 121/126 (96%) of the cases, the rest were counter-clockwise. Location of the pregnant horn and its relation to the direction of uterine torsion were determined in 39 buffaloes. Pregnancy was observed more frequently in the right horn (32/39, 81.1%), and the uterus twisted predominantly toward the pregnant horn (33/39, 84.6%). Vaginal delivery was possible after slow rolling of the mother in 65/126 (51.6%) of the cases. Cesarean section was performed after failed detorsion attempts or due to failure of the cervix to dilate following successful correction of the torsion in 12/126 (9.5%) of the cases. Cesarean section was performed immediately in 49/126 (38.9%) of the cases. Several risk factors were identified that increased the risk of fetal and maternal mortalities. The overall fetal mortality rate was 99/126 (78.6%). It was greater in high torsion (66/66, 100%) and that of moderate degree (30/39, 76.9%) than in torsion of mild degree (3/21, 14.3%). The odds of fetal death were 0.003 in mild torsion and 0.05 in moderate torsion compared to high torsion. Fetal mortality was lower when the patient was treated within 24 h of appearance of symptoms (6/17, 35.3%) and high when uterine torsion occurred before term (68/74, 91.9%). The odds ratio in favor of death was 0.09 in those cow treated within 24 h versus those treated later. Fetal mortality was greater when uterine torsion occurred during late pregnancy (68/74, 91.9%) than when it occurred at term (31/52, 59.6%). The odds ratio of

dying was 0.13 at full term compared to late pregnancy. Parity and method of treatment were non significant risk factors for fetal mortality. Fetuses were found emphysematous in 21/126 (16.4%) of the cases. The overall maternal mortality rate was 30/126 (23.8%). It was greater in torsion of high degree (22/66, 33.3%), than in torsion of moderate (7/39, 18%) and mild degrees (1/21, 8.3%). The odds ratios of death were 0.1 in mild degree and 0.2 in moderate degree compared to high degree. The stage of gestation, parity, duration of torsion and method of treatment were not risk factors. Mortality rate was greater in dams with dead fetuses (28/98, 28.6%) than in dams with alive ones (2/28, 7.1%). The odds ratio of maternal death was 0.3 in those with live fetuses versus those with dead fetuses. Maternal deaths were due to rupture of the uterus or cervix (14/30, 46.7%), toxemia (13/30, 43.3%), or due to indefinite cause (3/30, 10.0%). Maternal deaths occurred on the same day of treatment in 8/30 (26.7%) of the cases, the rest died after 48 h of the treatment. Head deviation and/or limb flexion was the commonest fetal cause of dystocia in buffalo (7.5% and 16.9%). Fetal anomalies as a cause of dystocia have been reported in buffaloes. Failure of uterine expulsive forces (Uterine Inertia) and neoplasms of vagina, vulva and uterus are also seen in buffaloes. Two buffaloes with full-term pregnancy suffered from dystocia because the cervix did not dilate in spite of strong labour pains and other parturition signs shown by each animal. The urinary bladder, cervix, vagina and surrounding area were very firm. Dead, emphysemated fetuses were removed by caesarean in each case and anuria was also noticed. One buffalo died and the other was euthanised after surgery because it did not improve. The post-mortem examinations revealed transitional cell carcinoma of urinary bladder infiltrating the cervix, vagina and surrounding area in each case.

Incidence of calving disorders is affected by buffalo production system, type of housing, nutrition, body condition, age, parity, milk yield, sex and size of calf. The deficiency of some elements e.g Calcium, Phosphorus, Copper, Iron, Zinc and Selenium appears to increase the incidence of calving disorders while macro and micro elements in soil and fodder also varied in three agro-ecological zones. It may be recommended that the excess feeding, underfeeding and feeding of imbalanced rations should be avoided to reduce the occurrence of calving disorders. The improvement in small managemental changes in the housing of the animals like provision of even floors is likely to reduce the occurrence of calving disorders.

The mineral supplements should be prepared and must be zone specific and tried in the field for recommendation to the farmers. The interrelationships of macro and micro mineral nutrients of soil, fodder and the animal needs to be investigated for each agro-ecological zone

9.5. Dystocia in Ewes and Does

Dystocia is a contributory factor in perinatal death of dams and newborns because of damage to the birth canal and use of excessive traction forces. Fetal dystocia occurred mainly due to oversize, mal-disposition, and monsters. Maternal dystocia were mainly due to a deficient dilatation of the cervical canal (ringwomb), narrow pelvis and uterine inertia.

Ringwomb, or incomplete cervical dilatation, is a problem in some sheep and goats flocks. It accounted for an incidence of 20 to 30% of all dystocia cases. The cause is still unknown but may be due to a lack of release of hormones involved in softening collagen or a lack of response of the collagen in the cervix to hormonal stimulation. There was no correlation of ringwomb with breed of sheep, age, and body condition score. However, there was a genetic predisposition; the occurrence of ringwomb appears to run with bloodlines and when these bloodlines are inbred, the frequency of ringwomb increases. Manual dilatation, medical and hormonal therapies, and surgical approach have been used to treat the ringwomb syndrome with variable outcomes. The administration of PGE_2, in form of intra-cervical gel, had no effect in causing softening of the cervix. Using relaxin on ewes affected with ringwomb did not appear to be of practical value. Treatment with calcium borogluconate and/or estradiol benzoate has shown satisfactory results in goats. However, the optimal response has been obtained by intramuscular injection of prostaglandin $F_2\alpha$ analogue.

In a study encountered by the author on two breeds of sheep (Awassi, *n*=161 and Najdi, *n*=19) and goats (Aardi, *n*=265 and Damascus, *n*=19) suffering from dystocia. Animals' age ranged from 13 months to 8 years and parity was from 1 to 6. The majority of animals were housed under free condition in the desert area of Qassim-KSA region and fed mainly on alfalfa hay and concentrate (barely seeds). Ringwomb, narrow pelvis, fetal mal-disposition, and fetal oversize were the most important causes of dystocia. Other less important causes were uterine torsion, monsters and simultaneous presentation of twins Monsters included schistosomus reflexus (four cases), bulldog lambs (three cases), and deformity of vertebral column and extremities (one case). Maternal dystocia occurred more frequently than fetal dystocia, both in Awassi and Najdi breeds as well as in primiparous. In multiparous, fetal dystocia was more frequent than maternal dystocia. On the other hand, ringwomb, fetal mal-disposition, narrow pelvis, and simultaneous presentation of twins were the major causes of dystocia. Other minor causes were uterine inertia, fetal oversize, uterine torsion, and monsters. Monsters constituted schistosomus reflexus (one case) and dicephalus twin (one case), general ankylosis and muscle contractures (one case), and failure of closure of abdominal wall (one case). Maternal dystocia occurred more commonly than fetal dystocia, both in Aardi and Damascus breeds and in primiparous. In multiparous, the difference between the frequencies of maternal and fetal dystocia was not significant.

Of the cases with ringwomb, cesarean section was performed promptly in 25/57 ewes and in 42/93 does to save the fetal live. The rests with dead fetus(es) were treated with $PGF_2\alpha$. After administration of PGF2α, 7/32 (21.9%) ewes and 35/51(68.6%) does had fully dilated cervix within 63.0±11.6 and 41.5±13.1 hr of treatment, respectively. The differences between ewes and does in response to the $PGF_2\alpha$-treatment and for the time needed for cervical dilatation were significant. Cases, which failed to response to $PGF_2\alpha$-treated, were immediately cesarean sectioned. During cesarean section for cases with ringwomb, pre-cervical uterine torsion (twisting anterior to the cervix) was found in three ewes and two does. These cases could not be diagnosed as having uterine torsion until they were surgically operated. Three cases of them showed completely ischemic uteri that underwent obligatory ovario-hysterectomy.

Suggested Readings

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APPENDIX:Tables and Figures

Table 16: Causes of dystocia in female camels (*n*=60)

Cause of dystocia	incidence
Uterine torsion	33.3%
Head deviation	11.7%
Big-sized fetus	10%
Narrow cervix	8.3%
Breech presentation/hock flexion	8.3%
Prolapsed vagina	5%
Dog sitting presentation	3.3%
Carpal flexion	3.3%
Shoulder flexion	1.7%
Transverse presentation	1.7%

Table 17: Causes of dystocia in mares

Cause of dystocia	Frequency
Abnormal posture especially of head and neck	**common**
Dorso-iliac and dorso-pubic position	**occasionally observed**
Wry neck	**common**
Transverse ventral presentation	**not uncommon**
Transverse dorsal	**rare**
monster	**Rare**
Disproportion between fetal size and pelvic diameter	**rare**

Table 18: Causes of dystocia in cows

Cause of dystocia	Frequency
Disproportion between fetal size and pelvic diameter especially in heifers	common
Fetal gigantism, hydropsy of the fetal membranes and fetus, and fetal emphysema	not uncommon
Monsters	higher than other species
Uterine inertia, failure of the cervix to dilate	occasionally noted
Twinning	common
Uterine torsion	common
monster	

Table 19: Causes of dystocia in buffalo-cows (*n*=143)

Causes	Incidence
Uterine Torsions	**49%**
Carpal flexion	**14.7%**
Head deviation	**13.3%**
Narrow cervix	**7.7%**
Fetal Emphysema	**4.9%**
Hock flexion	**3.5%**
Uterine Inertia	**1.4%**
Fetal Ascites	**2.8%**
Pelvic Fractures	**1.4%**
Fetal Monsters	**1.4%**
Breech presentation	**1.4%**

Table 20: Causes of dystocia in ewes (*n*=180)

Cause of dystocia	incidence
Narrow cervix	31.7%
Narrow pelvis	21.7%
Big-sized fetus	15%
Head deviation	1.7%
Hock flexion	4.4%
Uterine torsion	4.4%
Monsters	4.4%
Carpal flexion	2.8%
Shoulder flexion	2.2%
Twins	1.7%

Table 21: Causes of dystocia in does (*n*=284)

Cause of dystocia	incidence
Narrow cervix	32.8%
Narrow pelvis	18%
Twins	13.7%
Head deviation	13%
Uterine inertia	6.7%
Hock flexion	3.9%
Carpal flexion	2.5%
Uterine torsion	1.8%
Shoulder flexion	1.8%
Dog-sitting presentation	1.4%
Monsters	1.4%

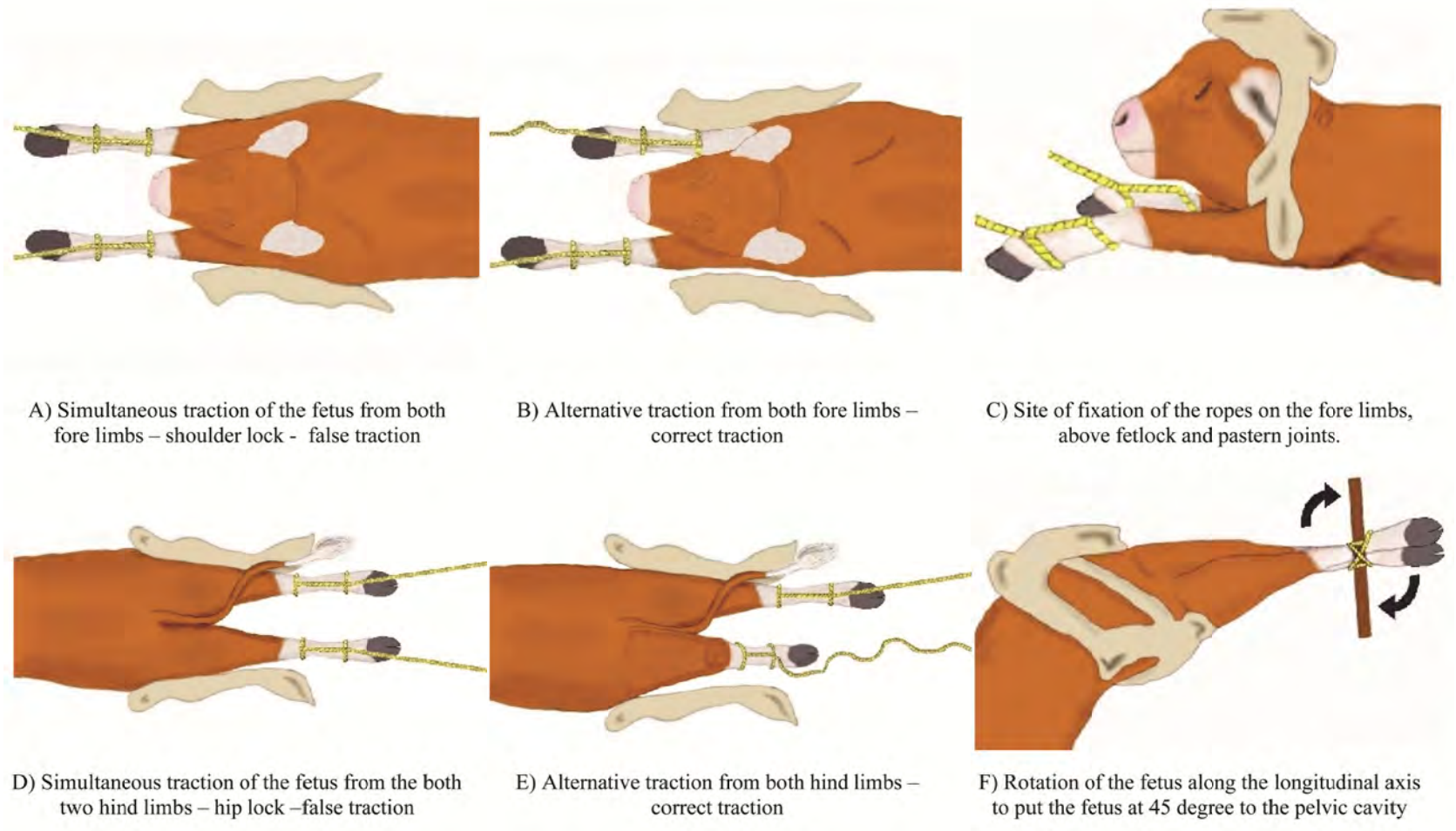
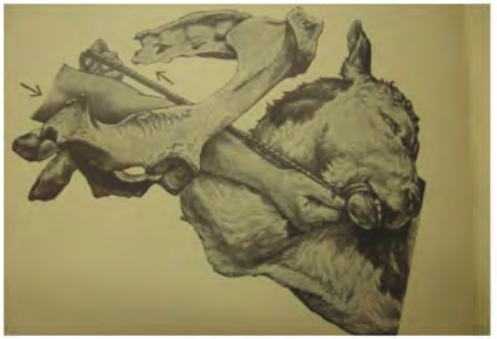


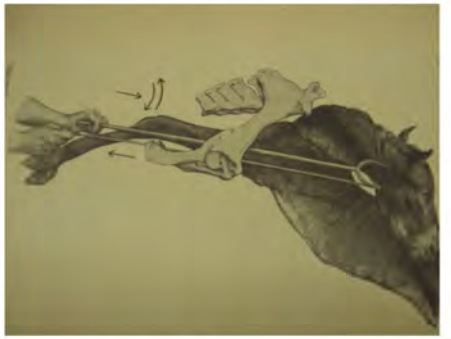
Fig. 57: Traction of over-sized fetuses in cattle (*www. animalsciences.missouri.edu*).

Fig. 58: Forced traction of a relative big-sized fetus in cattle (*Ohio-USA, 2005*).

A) Correction of laterally deviated head: using the operator's hand to protect the fetal teeth and rotating the head medially toward the fetal body



B) Correction of laterally deviated head: using a rope fixed in the fetal lower jaw to rotate the head by external assistance



C) Correction of laterally deviated head: using a Kuhn's crutch to rotate the head by an external help

A) Light degree of downward head deviation

B) Chin-breast posture

C) Using Kuhn's crutch to lift the head on the pelvis and pushing one of the fore limbs into the uterus to get space to correct the deviated head

Fig. 59: Correction of lateral and downward deviated head (*Arthur, 1964*).

A) Correction of the double hock flexion: a rope is used to lift the pastern on the maternal pelvis, simultaneously the obstetrician push the metacarpus forward and laterally inside the roomy uterus.

B) Correction of abnormal position: the operator fixed the two hind limbs by a rope and put a wood between the tied legs and rotate it to the required position.

Fig. 60: Correction in posterior presentation (*Arthur, 1964*).

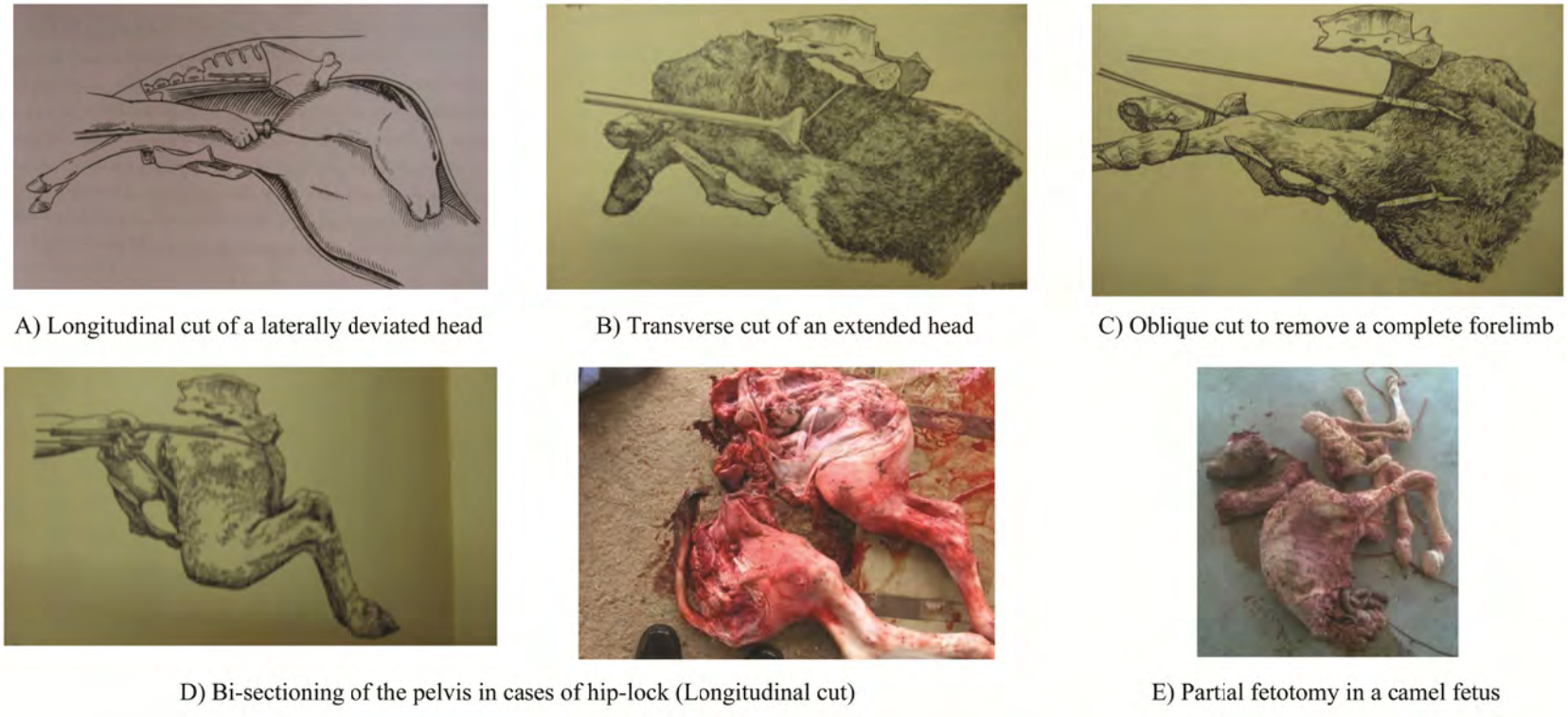
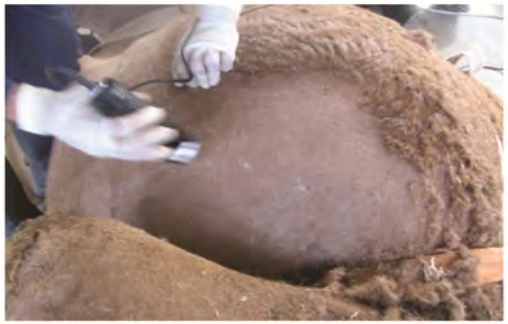
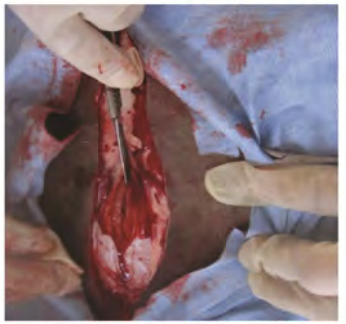


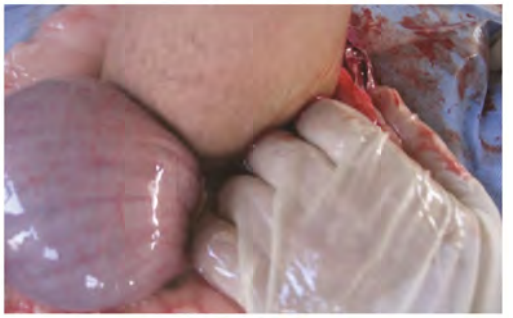
Fig. 61: Fetotomy in farm animals (*Arthur, 1964; Noakes et al., 2001; Qassim-KSA, 2007-2013*).



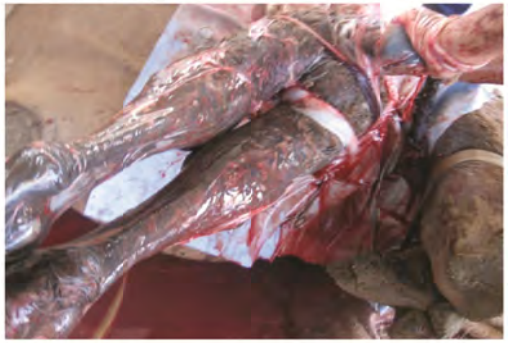
A) Sharing the hair at left flank region



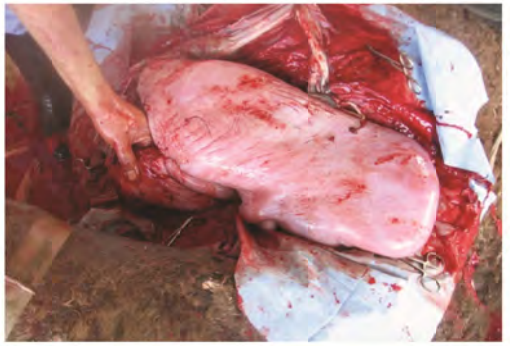
B) incising in the skin and muscles



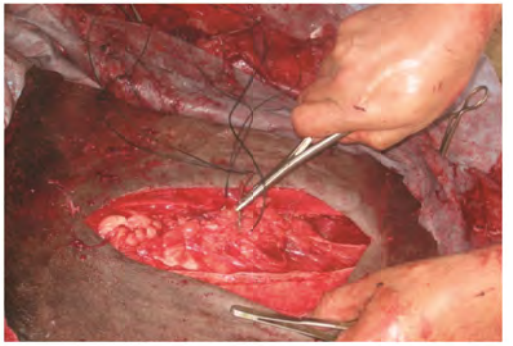
C) Lifting the uterus into the incision



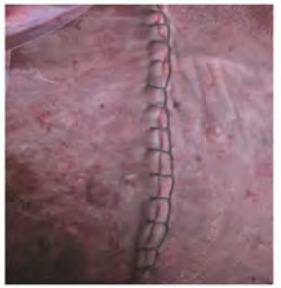
D) Traction of the fetus



E) Suturing the uterus



F) Suturing the muscular layers



G) Suturing the skin

Fig. 62: Cesarean section in camels – recumbent left flank approach (*Qassim-KSA, 2009*).

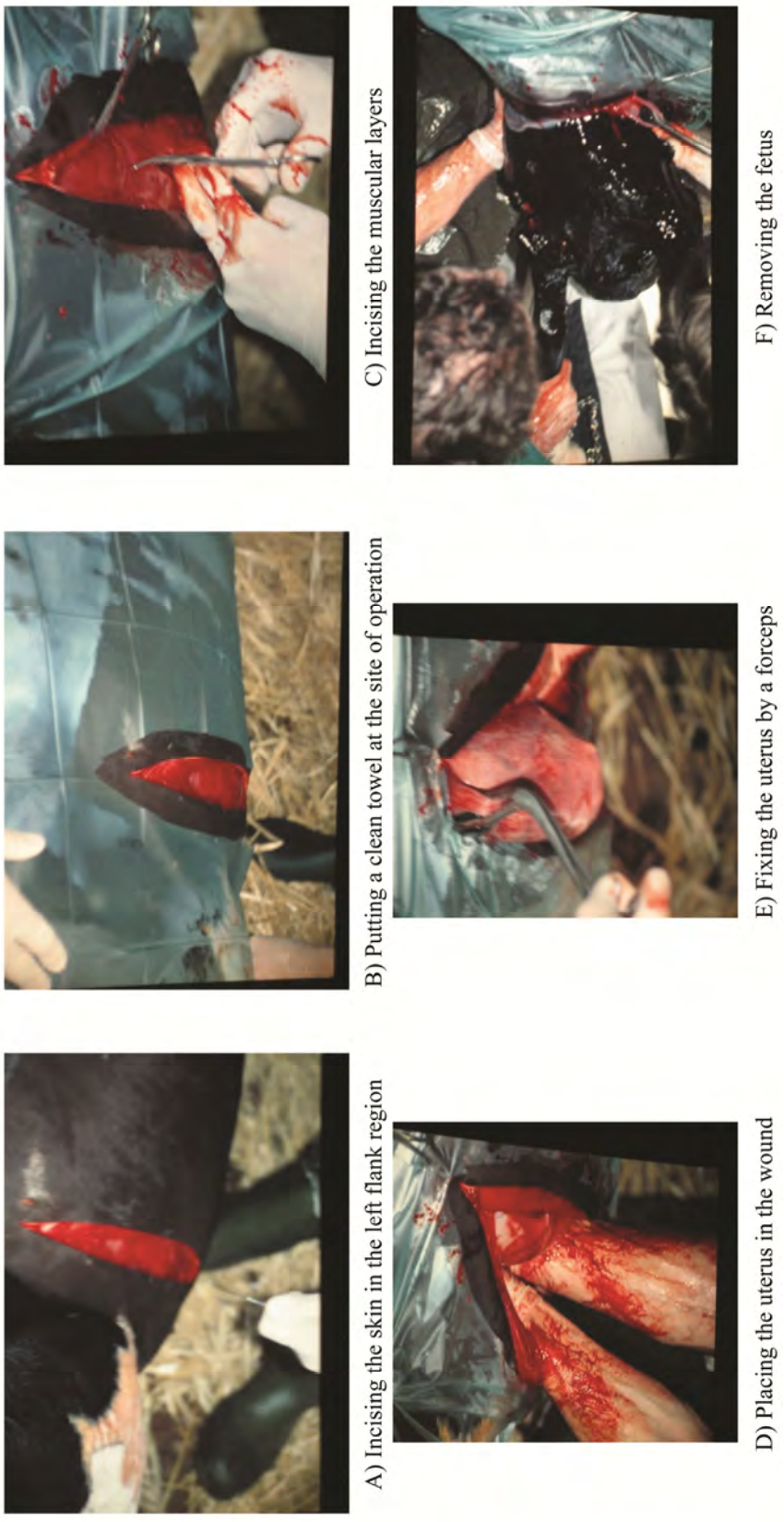


Fig. 63: Cesarean section in cattle (*Berlin-Germany, 1999*).

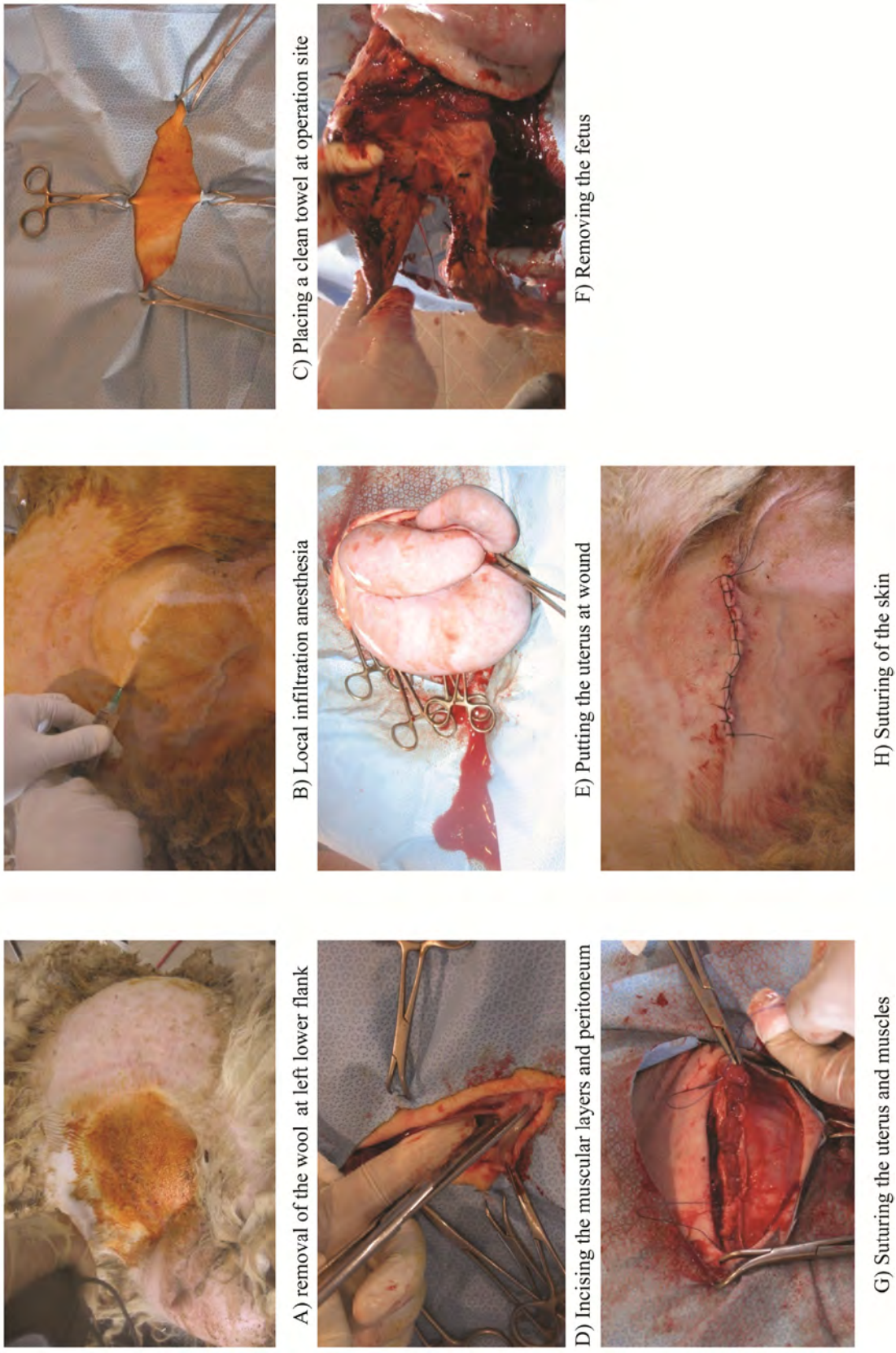
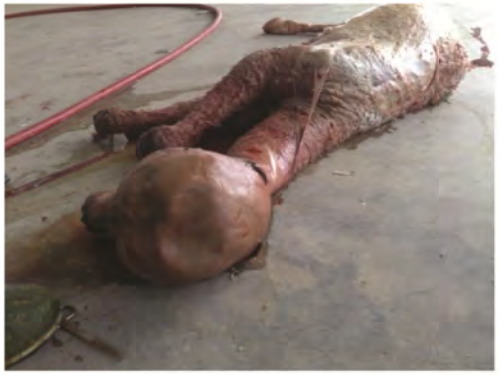
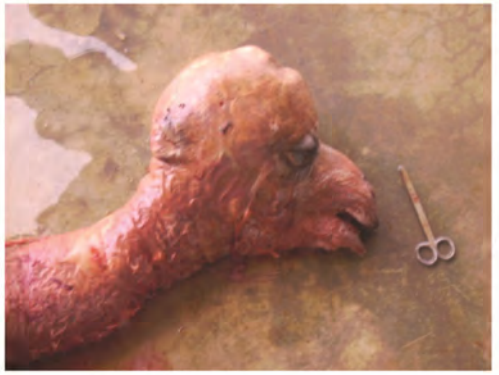


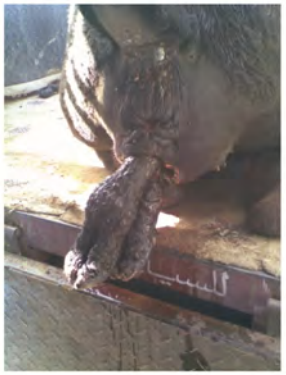
Fig. 64: Cesarean section in sheep (*Qassim-KSA, 2010*).



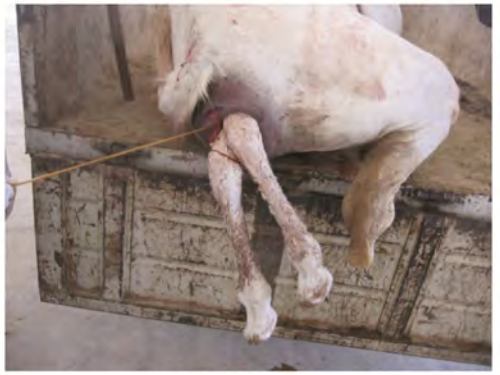


A) Large sized head (Hydrocephalus)

B) Retention of the two fore limbs (double shoulder flexion



C) Downward deviation of the head



D) lateral deviation of the head

Fig. 65: Dystocia in female camels (*Qassim-KSA, 2007-2013*).

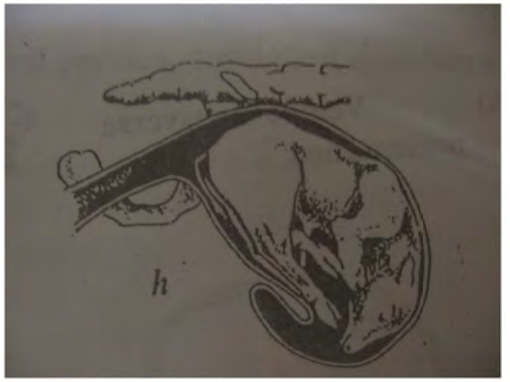
Fig. 66: Dystocia in she-donkeys and mares (*Arthur, 1964; Assiut-Egypt, 2003; Qassim-KSA, 2007*).

A) Big – sized fetus

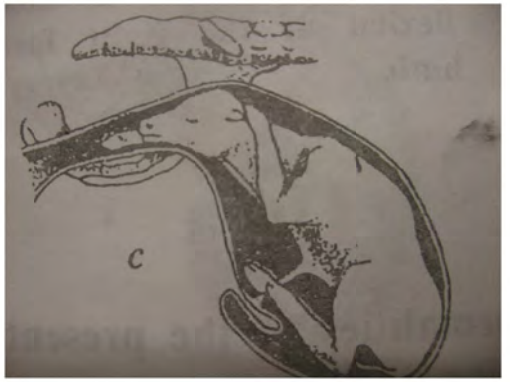
B) Fetal Ascitis

C) Lateral deviation of head

D) Downward deviation of the head



E) Breech presentation



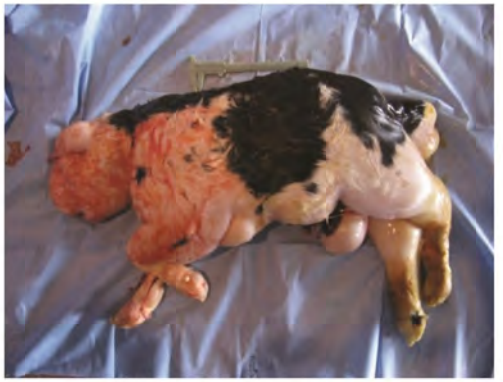
F) Foote-nape posture

Fig. 67: Dystocia in cows (*Roberts, 1986; Assiut-Egypt, 1988-2007*).

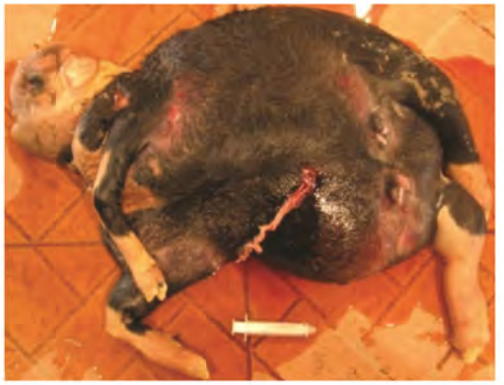
B) A fetus with hydrocephalus

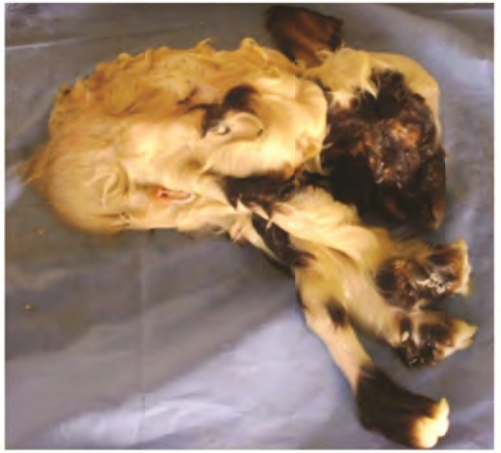
A) An over-sized fetus (emphysematous fetus)

Fig. 68: Dystocia in buffaloes (*Assiut-Egypt, 2003*).



A) Bull-dog lambs (general anasarca)



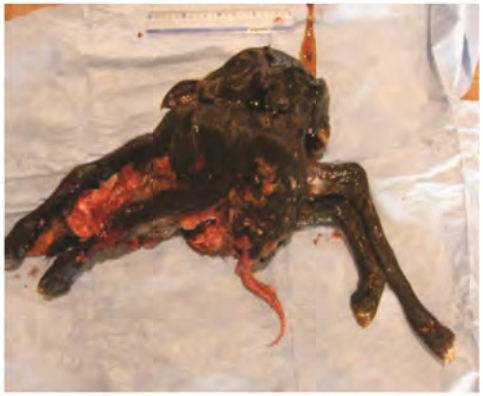


B) Persomus elumbis (Awassi breed)

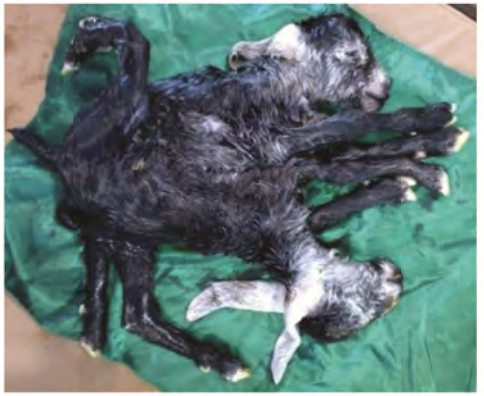
C) Arthrogryposis (Najdi breed)

D) Dog-sitting position: the head and two hind limbs appear altogether.

Fig. 69: Dystocia due to fetal anomalies in sheep (*Qassim-KSA, 2007-2013*).



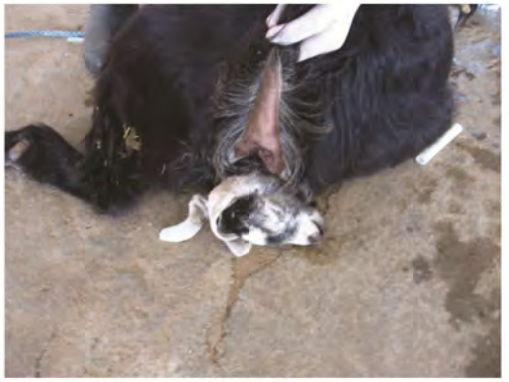
A) Schistosomus reflexus (Damascus breed)



B) Dicephalus twin (Aardi breed)

C) Arthrogryposis and Lordosis (Aardi breed)

D) Failure of closure of abdominal wall and exposure of abdominal organs (Aardi breed)



E) Double shoulder flexion (Aardi breed)

F) Deviation of the head (Aardi breed).

Fig. 70: Dystocia in goats (*Qassim-KSA, 2007-2013*).

Chapter 10

Postpartum Period

Postpartum or postnatal (Latin for after birth, from post, meaning after, and natalis, meaning of birth) is the period beginning immediately after the birth and extending for about six weeks or until the body has completed its adjustments and has returned to the pre-pregnant state. The term postpartum period refers to the mother (whereas postnatal refers to the infant). Less frequently used is puerperium. It is the time after birth, a time in which the mother's body, including hormone levels and uterus size, returns to a non-pregnant state. Lochia is post-partum vaginal discharge, containing blood, mucus, and placental tissue.

This period from birth until the mother conceives is the most critical period in an animal's production cycle and minimizing this time period is important for several reasons. Animals that cycle early in the breeding season have higher conception rates than those that cycle later for several reasons one of the most important is that the animal that cycles earlier has more chances of getting pregnant during a limited breeding season. Nutrition the last 50-60 days prior to birth has a profound effect on cyclicity after calving; this is further exaggerated by body condition of the mother. Animal in moderate body condition that loses weight before birth is much more vulnerable to postpartum nutrient levels than one in similar or even poorer condition that is gaining weight prior to birth. Nutrition pre- and post calving is of critical importance to the postpartum interval. Body condition, level of nutrition, age, milk production, weather, disease, parasites, and other factors will affect the ability to shorten the postpartum interval. Nutrition is probably the easiest way to shorten the postpartum period.

The characteristics of the postpartum period in farm animals and the common postpartum complications are shown in Table (22) and Figs. (71-76).

10.1. Postpartum Period in Female Camels

10.1.1. Physiology of the postpartum period

Uterine involution is completed from 25 to 30 days postpartum and follicles (≥1.0 cm diameter) could be found from 34 to 70 days postpartum. Females may be conceived when mated by the end of this time.

Progesterone concentrations in the dams varied between 0.5 and 2.0 ng/ml on the day of calving and declined steadily thereafter to become undetectable by Day 9; progesterone remained undetectable in the neonates. Cortisol concentrations were high (25 to 30 ng/ml) at parturition in both the dams and their calves. They declined to 6 to 7 ng/ml in the dams, but became undetectable in the neonates by Day 14 post partum. The thyroid hormones were low in the dams (T4 = 70; T3 = 1.6 ng/ml) on Day 1 post partum but then increased gradually to Day 21 (T4 = 110; T3 = 2.2 ng/ml). In contrast, thyroid hormone concentrations in the neonates were 4 to 5 times higher than those of their mothers at birth. They declined thereafter but nevertheless remained at almost double the concentrations found in the dams.

In a study of the authors on dromedary camel during the postpartum period, it was found that the mean intervals for complete involution of the previously-pregnant horn, non-pregnant horn, and cervix were 34.33 ± 3.9, 29.01 ± 0.81, and 28.71 ± 1.51 d, respectively. Involution was rapid between Days 3 and 17 postpartum, and slow from Day 17 onwards. Regression rates of the cervix, non-gravid and gravid horns were 1.44 ± 0.41, 1.46 ± 0.41 and 1.71 ± 0.64 cm/d, respectively. Both ovaries were accessible for examination between Days 10 and 17 postpartum in five of the six female camels, and by Day 24 in one of the six. Recruitment of a new follicular growth (≥ 4 mm in diameter) was observed between Days 14 and 17 postpartum. Recruitment was first observed on the ovary contra-lateral to the previously-pregnant horn in all but one case. Only one follicle reached the ovulatory size (≥ 0.9 mm in diameter) between Days 17 and 31 postpartum in all camels except one, which showed two large follicles (1.1 and 1.5 cm) on Day 24 postpartum. Development of the medium and large-sized follicles occurred at the cost of the small ones. All females, except one, ovulated as a result of the GnRH agonist treatment. Ovulation occurred within 72 h of treatment. The mean size of the ovulatory follicle at the time of the GnRH treatment was 1.17 ± 0.3 cm (range, 0.9 to 1.5 cm). In one female, which had 2 large follicles at GnRH treatment, only one follicle ovulated while the other developed until it reached a diameter of 2.7 cm before it started regression. The CL was firstly detected by Day 4.1 ± 1.6 after GnRH treatment. It reached a mean maximum diameter of 2.1 ± 0.5 cm by Day 10.2 ± 0.4 post-treatment and lasted for a mean duration of 6 ±1.1 d. On ultrasound examination, 30 d after mating, two of the five females were found to be pregnant. The other three females conceived on the second service. No fetal losses were detected on subsequent examinations. The mean interval from calving to conception (open days) was 78.1 ± 3.71 d. Serum P4 concentration remained basal during the postpartum period and increased only after GnRH treatment. It began to increase to a mean level of 1.24 ± 0.9 ng/mL by a mean time of 6.2 ± 1.8 d (range, 3 to 7 d) post-GnRH treatment. Serum P4 reached a mean maximum level of 3.24 ± 3ng/mL by a mean time of 9 ± 1.9 d (range, 7 to 11 d) after treatment, and then began to decrease gradually to a basal level. Serum E2 peaked when a large follicle (≥0.9 cm in diameter) was observed on the ovary. It peaked again in a mean time of 8.5 ± 2.8 d (range, 3 to 11 d) after GnRH treatment. Except for a slight and non- significant gradual increase, serum T4 did not show any specific pattern during the observation period. Serum FSH exhibited a biphasic pattern with two noticeable peaks: one just before or coinciding with the recruitment of small follicles and another major one, 4.67 ± 4.1 d (range, 3 to 13 d) after GnRH treatment.

10.1.2. Pathology of the postpartum period

The most common disorders of the reproductive organs in the postparturient camelids are retained placenta, postpartum metritis and uterine prolapse.

10.1.2.1. Uterine prolapse

Uterine prolapse occurs as a complication of parturition especially after dystocia, retained placenta or excessive obstetrical manipulation. Uterine prolapse occurs in old females with excessively relaxed vagina and pelvic ligaments. The prognosis depends on the extent, the state of the uterine tissue and presence of other complications such as toxic shock and ruptured uterine blood vessels. The prognosis is poor if there is evidence of uterine blood vessel rupture, lacerations or necrosis due to prolonged exposition of the uterus to the harsh environment (cold or hot). Replacement of a complete uterine prolapse in the female dromedary is very challenging and requires use of sedation.

10.1.2.2. Retained placenta

In the dromedary, delivery of the placenta can take up to 12 hours with no effect on the dam. The incidence of retained placenta is increased following dystocia and cesarean section. Most cases of retained placenta are due to uterine inertia. In some cases, a retained placenta may require manual removal. The placenta removed in this manner should be spread to verify if it is complete. In the case of partial retention of the placenta or evidence of placentitis, an antibiotic treatment should be given as soon as possible to prevent development of septic metritis. Other treatments for retained placenta used in equines include distention of the uterus with warm, dilute povidone iodine solution or injection of oxytocin (30 to 40 IU IM) to promote uterine contraction.

10.1.2.3. Postpartum infection

During the postpartum period, the uterus is exposed to bacterial contamination. Prevention of postpartum metritis can be achieved by regular monitoring of the periparturient female, hygienic conditions during parturition and early and efficient obstetrical manipulation if needed. Any manipulation of the genital organs should be followed by local or systemic prophylactic antibiotic treatment. All postparturient females should be monitored for signs of fever or septicemia for the first 5 days postpartum.

10.1.2.4. Perineal laceration

Perineal or recto-vaginal lacerations occur because of pressure of the fetal forelimb or forceful traction of the fetus. Perineal laceration does not necessarily affect the fertility of the female if the vestibular sphincter is preserved and prevents entry of fecal material into the vagina. However, mating should be carefully monitored in order to prevent intromission of the penis into the rectum. To prevent these problems surgical repair should be attempted when the wound has healed and swelling and granulation tissue have subsided.

10.2. Postpartum Period in Mares

10.2.1. Physiology of the postpartum period

The mare is unusual compared with many other domestic species in that uterine involution is extremely rapid, and there is a return to fertile estrus within a few weeks of parturition. A new pregnancy may be established very early in the post-partum period. Myometrial contractility increases after parturition and is greater under the influence of estrogen when the mare returns to estrus. Uterine involution is amazingly rapid after normal parturition. Histologically, there is no disruption of the endometrium at parturition. The uterine horn which housed the fetus will remain larger than the other horn. It may be difficult to define when involution is complete, i.e. when the previously-pregnant horn is no longer identifiable by palpation. The cervix remains relaxed until after the foal-heat ovulation. New pregnancies almost invariably establish in the smaller uterine horn (previously non-gravid). There is uterine tone during involution and after the foal heat. This may make early manual diagnosis of pregnancy difficult; enlargement at the base of the previously-pregnant horn may be mistaken for an 18–28 day conceptus.

In an endocrinological study, Plasma concentrations of immunoreactive (ir-) inhibin were measured in nine pregnant mares 30 days before foaling till Day 7 after ovulation of the first post-foaling estrus (foal heat) using a heterologous bovine-based radioimmunoassay (RIA). Other hormones like IGF-I, FSH, LH, Estrogen and progesterone were also measured during the same period. During the post partum period and foal heat, the resumption of ovarian cyclicity was estimated ultrasonically on 36 animals. Most animals resumed ovarian cyclicity by the 5th day (13.88%), 7th day (47.22%), 9th d postpartum (30.55%) and the remaining animals showed estrus on variable times from the 10th to the 13th day post-foaling (8.38%). Ovulation occurred on the 9th day in 3 mares (8.33%), 11th day in 6 mares (16.6%), 13th day in 19 mares (52.77) and after the 13th day in 8 mares (22.2%). During the foal heat, most activities were observed from the left side (63.88%). A marked increase in plasma concentrations of ir-inhibin was observed during the last month of pregnancy with two noticeable peaks, the first occurred 19 days prior to parturition and the second took place 6 days pre-foaling then the level fell gradually as foaling approached. Post-foaling, immunoreactive-inhibin increased concomitant with the resumption of the ovarian activity. LH did not show significant changes during the last month of pregnancy whereas there were two distinct peaks of FSH level in the last week of pregnancy with 6 days apart. The first peak found 7 days before foaling while the second rise was noticed on the day of foaling. FSH and ir-inhibin patterns were inversely correlated. It was suggested that, FSH increase prior to foaling may be responsible for the rapid resumption of ovarian activity in mares and initiation of foal heat. Progesterone concentrations showed a biphasic pattern during the last month of pregnancy with two peaks; 20 days and 3 days. During the post-partum period, progesterone decreased significantly to its nadir till the first ovulation post-foaling. Plasma IGF-I concentrations increased greatly during the last three weeks of gestation in all the studied mares. Their peak levels were noticed during the day of foaling (531.55 ± 34.52 ng/ml). However the level showed steady pattern after foaling. Estradiol-17β concentrations decreased gradually during the last month of pregnancy and reached its lowest value by the day of parturition. Its concentrations decreased dramatically during the early post-partum period and started to increase on the 6th day

postpartum with the resumption of the ovarian activity (foal heat). After ovulation, E2 level dropped to its basic values.

Most mares usually return to estrus approximately 5–9 days after parturition. This estrus is generally known as the 'foal heat', since the foal often develops a physiological scour at this heat. Fertility at the first post-partum estrus has been recorded as being 5–10% lowers than at subsequent estruses. This may be related to a failure of the uterus to become completely involuted.

Bacteria may enter the mare's uterus post partum. This risk can be reduced by suturing or clipping the dorsal vulva closed immediately after delivery. The post-partum uterine flora is usually dominated initially by coliformis, and later by β-haemolytic streptococci. Post-partum colonization of the uterus by bacteria is a normal event, and it should be expected. After normal parturition, most mares eliminate bacteria before the foal heat. For the first few days after parturition there is a moderate volume of vulval discharge (lochial fluid expelled from the uterus). Very little discharge is normally seen after the first few days post partum.

10.2.2. Pathology of the postpartum period

10.2.2.1. Postaprtum infection

Post-partum infection of any significance is associated with uterine luminal fluid that can be detected using ultrasound imaging. Persistent vulval (cervical) discharge is indicative of infection. Uterine swabs may be investigated for the presence of bacteria and/or neutrophils.

10.2.2.2. Uterine prolapse

Uterine prolapse is a relatively rare condition that is difficult to manage when countered in the mare. This mare's uterus had to be handled more gently than that of a cow. Had the mare remained standing, it would have been necessary to raise the uterus on a piece of plywood or a large towel rather than in ones hands, to avoid damage to the endometrium. Sedation and good restraint (possibly breeding hobbles) would also be required to prevent her kicking the uterus. There seemed to be fewer problems once the mare was down. Epidural anesthesia is advised as the best method to prevent straining by the mare. Possibly the epidural anesthesia could be increased enough to give hind leg paresis if other methods of restraint proved inadequate. The likelihood of recurrence after proper replacement is slight. Metritis and laminitis were major concerns after replacing the uterus. The intrauterine infusion of 1000 mL 50% dextrose and 250 mL 0.2% nitrofurazone was intended to flush out any remaining debris and to prevent infection. This amount of fluid should also help to straighten the organ fully to the normal position. Uterine tone and involution are encouraged by this type of infusion as well. Penicillin-streptomycin daily for five days was also used to prevent metritis. The antihistamine injections every twelve hours and checking the mare's feet were the steps taken to avoid the complication of laminitis. A tetanus vaccination is also indicated after replacing a prolapsed uterus in the mare.

10.2.2.3. Perineal rupture

Perineal lacerations most commonly happen to mares during foaling. They are graded according to the depth and severity of the tearing sustained. Mares should be surgically treated (see surgical section) by local wound treatment, antibiotics and anti-inflammatories.

10.2.2.4. Uterine rupture

Uterine rupture was diagnosed in 2 postpartum mares with hemorrhagic vaginal discharge. Both mares had abdominal pain, as evidenced by pawing, kicking at the abdomen, or attempting to roll. Peritoneal fluid analysis was useful in establishing a diagnosis.

10.2.2.5. Retained placenta

The average time needed for placental expulsion is about one hour and should not take more than two hours. Most clinicians consider the fetal membranes to be retained if they are not passed within three hours of birth. Retention of the fetal membranes (RFM) is one of the most common peripartum problems in the mare, with an incidence in the range from 2% to 10%. Complications include acute metritis, septicaemia, laminitis and even death. With prompt and effective treatment these sequelae can be avoided. In many cases, uterine involution is delayed even if these more serious complications do not develop. The riding horses and ponies of today are less likely to suffer from these complications, but RFM should be treated as an emergency.

The precise cause of retained fetal membranes remains unclear. The most likely is uterine inertia due to hormonal imbalance. Oxytocin has an important role in postpartum uterine contractions and low levels of this hormone in the circulation may result in abnormal myometrial activity. This in turn leads to placental retention. The incidence of RFM is much higher incidence after dystocia, which is probably due to either uterine trauma or uterine inertia.

The most obvious sign of a retained placenta is the appearance of a variable portion of the placenta protruding from the vulval opening. Less commonly no placenta may be visible. This either means that no part of the placenta has been passed or, more likely, portion of the placenta remains attached. The placenta should initially be tied up in order to prevent touching the hocks. As uterine contractility plays an important role in the dehiscence of the fetal membranes administration of oxytocin is recommended as a first and many times (up to 90% of cases) successful treatment. It is a good rule not to wait longer than 6 hours after delivery (depending of the case history or in heavy breeds earlier is preferable) before such a treatment is started. This treatment avoids manipulation in the uterus with the risk of introducing microorganisms. Oxytocin can be given via the intramuscular route (20-40 IU), which can be repeated after one hour if the placenta has not been passed. Alternatively, use slow intravenous infusion of 50 IU oxytocin in 1 litre of physiologic saline during one hour. Symptoms of colic often follow injections of oxytocin and commonly precede natural expulsion so that pain-relieving drugs and sedation may be required. Only if this treatment fails and the placenta is almost detached but retained within the uterus should one attempt gentle manual removal. This interference should be carried out with scrupulous regard to asepsis, and no undue force should be applied, for even moderate traction on the afterbirth may cause the uterus to become

inverted and prolapsed. A third method described in the literature and which has may be successful under circumstances is the placement of some 10 L of warm, sterile saline inside the chorioallantoic membrane. Stretching of the uterine wall and stimulating uterine contractions via endogenous oxytocin may assist the separation of the microvilli from their endometrial crypts. This treatment should be used in combination with exogenous oxytocin administration. After removal, it is always important to check the placental membranes for completeness confirming that all the allantochorion has been removed. If pieces of membrane are left in the uterus they are usually impossible to reach due to the disproportionate size of the immediate post-partum uterus compared with the veterinarians arm. If necessary, the uterus should be flushed and siphoned to remove any fluid exudates remaining in the uterus by using a stomach tube and funnel. Aftercare includes (depending on the severity of the case) regular general examination, checking the uterus (for involution and contents) and, if indicated flushing and siphoning the uterus once or twice daily for a few days in combination with further injections of oxytocin.

10.3. Postpartum Period in Cows

10.3.1. Physiology of the postpartum period

Dairy cows resumed ovarian cycles (as determined by milk progesterone measurements) after 24.0 ± 0.6 days postpartum. Over 80% had resumed ovarian cycles by day 30 postpartum and 95% by day 60. In suckling beef cows, however, the time to resumption of ovarian cycles was 59.9 ± 2.5 days after calving and there was considerably more variation both within and between herds. Calf removal, either temporary or permanent, or the prevention of suckling by the fitting of nose-plates to the calves has been reported to shorten the acyclic period. Increasing the suckling intensity by double or multiple suckling (two or more calves per cow) has been reported to increase the postpartum acyclic period. Associations between high milk yield and reduced fertility have been reported for many years.

10.3.2. Pathology of the postpartum period

10.3.2.1. Retained placenta

Primary retention of the fetal membranes results from a lack of detachment from the maternal caruncles, whereas secondary retention is related to a mechanical difficulty in expelling already detached fetal membranes (e.g., uterine atony). Greater than three fourths of cows, however, expel their placenta by 6 hours and very few cows after 12 hours post partum. Detrimental effects on reproductive performance, milk production, postpartum disease, and culling rate were detected when duration of retention exceeded 12 hours.

Detachment of the fetal membranes indicates that uterine involution is progressing normally.

Although the main economic impact of RFM seems to be decreased milk production (more days open, decreased milk volume, milk from treated cows withheld), the correlation between RFM and mastitis is controversial.

The treatment objectives for RFM are to cause early detachment of the membranes in order to reduce the occurrence of metritis, decrease milk losses, reduce reproductive inefficiency, and decrease veterinary expenses.

Untreated cows more often are affected by endometritis and require repeat breeding than are treated cows. In a trial, uncomplicated retention did not affect the fertility of cows mated beyond 60 days of the last calving. In other studies, however, retention for more than 12 hours has been found to be detrimental to reproductive performance and milk production. Many clinicians believe that dairy cows with RFM should be treated, but the evidence regarding the advantage of treating RFM in beef cows is not conclusive.

Many approaches have been used to detach retained membranes. These include manual removal, attachment of a weight to the membranes to speed expulsion, electrical stimulation of the membranes with a pulse generator to initiate uterine motility, acupuncture to dilate the cervix, and administration of uterokinetic drugs, sulfonamides, prostaglandins, antibiotics, antiseptics, and hormones. None of these methods is effective in the treatment of RFM.

Manual removal of retained membranes is contraindicated because uterine infections are more frequent and more severe after this form of intervention than they are in untreated cows. Manual removal has been found to prolong the interval from calving to first functional corpus luteum by 20 days. When the placenta is not removed completely, a prolonged vaginal discharge follows. It is not easy to properly remove a retained placenta; 62% can be removed completely, 27% partially, and 11% are nonremovable. Often, attempts at removal during the first 48 hours after calving are unsuccessful because the placenta is too firmly attached and the apical part of the gravid horn is beyond the reach of the veterinarian. PGF2α does not cause detachment of retained membranes, significant uterokinesis in the early postpartum cow, or improvement of reproductive performance. Oxytocin is the uterokinetic hormone of choice in the early postpartum cow. In one study, 200 U given intramuscularly (IM) caused an almost immediate uterokinetic effect that lasted over 2 hours, with no spastic contractions. In another study, however, large doses of oxytocin were shown to create uterine spasm. Therefore, doses of 20U three to four times daily have been used. Intrauterine and systemic antibiotics do not hasten detachment of retained membranes. A new approach for the treatment of RFM is the injection of collagenase into the umbilical arteries retrieved from RFM. This approach may be superior to traditional treatments because it is specifically directed at correction of the lack of cotyledon proteolysis.

10.3.2.2. Bacterial contamination

Uterine health is often compromised in cattle because postpartum contamination of the uterine lumen by bacteria is ubiquitous, and pathogenic bacteria frequently persist causing clinical disease. The subfertility associated with uterine infection involves perturbation of the hypothalamus, pituitary and ovary, in addition to the direct effects on the uterus, and appears to persist even after clinical resolution of the disease. Examination of the contents of the vagina for the presence of pus is the most useful method for diagnosis of endometritis. The character and odor of the vaginal mucus can be

scored and this endometritis score is correlated with the growth density of pathogenic bacteria in the uterus, and is prognostic for the likely success of treatment.

Infection with one or more of *A. pyogenes*, *Escherichia coli*, *Pseudomonas spp*., *Streptococcus spp*., *Staphylococcus spp*., *Pasteurella multocida*, *Clostridium spp*., *Fusobacterium spp*. and Bacteroides spp. is common in the first 2 weeks postpartum. By 3– 4 weeks postpartum, the number of bacteria and the variety of species has diminished substantially in healthy cows. The significance of bacterial culture from the postpartum uterus depends on the species isolated and the interval since calving. The bacterium that is consistently associated with chronic uterine inflammation is *A. pyogenes*. This opportunistic, Gram-positive facultative anaerobe is commonly present in mixed culture with a wide variety of organisms, but most often with the anaerobes *Fusobacterium necrophorum* and *Prevotella melaninogenicus*, *E. coli* or *Streptococcus spp*. with which *A. pyogenes* acts synergistically. Recent research indicates that uterine infection predominated by *E. coli* in the first week postpartum and *A. pyogenes* in the second week is associated with subsequent endometritis. The presence of *A. pyogenes* beyond 3 weeks postpartum is associated with purulent vaginal discharge, persistent infection, elevated inflammation score in endometrial biopsies, and impaired reproductive performance. A wide variety of therapies for endometritis have been reported, including systemically or locally administered antibiotics, or systemically injected PGF2α. The general principle of therapy of endometritis is to reduce the load of pathogenic bacteria and enhance uterine defense and repair mechanisms, and thereby halt and reverse inflammatory changes that impair fertility. Intrauterine infusion of antimicrobials Infusion of antimicrobials into the uterus is aimed at achieving high concentrations at the site of infection. In contrast to systemic administration, intrauterine administration achieves higher drug concentration in the endometrium, but little penetration to deeper layers of the uterus or other genital tissues. Substances reported to be used for IU infusion include tetracycline, penicillin, cephapirin, chloramphenicol, diluted Lugol's iodine, gentamycin, spectinomycin, sulfonamides, nitrofurasone, iodine, and chlorhexidine. Lugol's iodine and oxytetracyline are irritating and are reported to cause coagulation necrosis of the endometrium. In field trials, IU infusion of antibiotics in a variety of protocols to treat endometritis has generally failed to show any benefit in reproductive performance over PGF2α.

10.3.2.3. Postpartum Bleeding

Postpartum bleeding results from ruptured of uterine/cervical/vaginal blood vessels resulting in massive blood loss. Although the incidence is not high, the cure rate is low because of its high mortality. It occurs mostly in primiparous beef cows when the body is not fully reached maturity and the birth canal is narrow. Fat cow has limited expansion of the birth canal, resulting in the birth canal stenosis. In addition, the movement of pregnant cows cannot be ignored, especially in late pregnancy, exercise is very important; otherwise it will increase the possibility of occurrence of dystocia. Fluid therapy, simulation of uterine contraction, and using vasoconstrictor like ergometrine are the first aid. The specific method is: the gauze around into a cylinder (a little thicker than the birth canal), then hand the oppression of gauze in the wound, and for 10 minutes to 20 minutes. Such as slow or stop bleeding can hand out, bleeding gauze to maintain 12-24 hours after the removal. If the injury site in

the vulva and vaginal vestibule can be taken to clamp or suture to stop bleeding. Antibacterial anti-inflammatory are used to prevent infection.

10.4. Postpartum Period in Buffalo-Cows

10.4.1. Physiology of the postpartum period

To maintain a calving interval of 13–14 months in buffaloes, successful breeding must take place within 85–115 days after calving. The postpartum period in the buffalo like the cow starts with parturition and ends with complete uterine involution and resumption of cyclic ovarian activity and normal estrous expression. Hormonal changes during the peri-parturient period besides regulating lactogenesis and parturition have their impact on postpartum reproductive activity.

The basal plasma levels of FSH in Murrah buffaloes exhibited a significant reduction from Days 60 to 240 of gestation. During the postpartum period, the baseline levels were 7±0.8, 11.8±1.7 and 12.0±1.8 ng/ml on Days 2, 20 and 35, respectively. On the contrary, no significant variations in plasma FSH levels between Days 3 and 90 or between milked (38.3±1.5 ng/ml) and suckled (35.7±1.5 ng/ml). Pituitary release of FSH in response to exogenous GnRH declined progressively with the advancement of pregnancy, but remained similar with no significant variations between Days 2, 20 and 35 postpartum. Availability of releasable FSH does not appear to be a limiting factor for resumption of estrous activity in the postpartum buffalo. Basal plasma LH concentrations in the buffalo did not vary between 60 and 240 days of gestation. During the last stages of pregnancy, LH values ranged between 0.4 and 0.9 ng/ml in dairy buffaloes. In postpartum buffaloes with no history of estrus or ovulation low serum LH levels were noted during the first 4 months (0.6±0.11 to 1.1±1.3 ng/ml) with no significant differences. Also no significant variations were observed in the basal LH levels between Days 3 through 90 postpartum or between milked (0.9±0.2 to 1.3±0.2 ng/ml) and suckled (0.9±0.1 to 1.5±0.2 ng/ml) anestrous Murrah buffaloes. The responsiveness of the pituitary gland to exogenous GnRH declined during pregnancy but was drastically increased between Days 2 and 20 postpartum. Hence it was hypothesized that the capability of the pituitary gland to respond to exogenous GnRH is restored by Day 20 postpartum in dairy buffaloes. A corresponding period of 30 days was noted in suckled swamp buffaloes. There is sufficient evidence that follicular activity is resumed early after parturition in the buffalo. A progressive increase of estradiol-17 was observed as early as 241–243 days of gestation (but marked increase of total estrogens and estradiol-17 occurred only in the last 15–5 days. Peak values of estradiol-17 of 142.0 pg/ml and 210±27 pg/ml were achieved either 1 or 2 days before parturition or on the day of calving. Also total estrogens reached their maximum values of 251±17 and 240±10 pg/ml in buffalo cows and heifers, respectively, at the day of calving. After parturition the mean plasma levels of estradiol-17 dropped steeply over the first 24–72 h. Basal values were reported between Days 2 and 7 after calving with minor fluctuations thereafter (11±3 to 18±3 pg/ml) until day 45 postpartum. A gradual increase of peripheral plasma concentrations of prostaglandin metabolite occurred over the last 15–7 days prepartum to reach peak values of 4±0.3 ng/ml on the day of calving. Higher concentrations of 14±2 ng/ml were reported during delivery which then dropped to 5–8 ng/ml during the first 6 days

postpartum. Lower values of 4±0.5 ng/ml on Day 1 after delivery and 1.3±0.2 ng/ml and 0.4±0.3 ng/ml on Day 3 were recorded. Basal values of 0.2 ng/ml and 0.14±0.05 ng/ml were reached on Days 15–20 and 22 postpartum, respectively. Knowledge of these changes is essential to understand the factors responsible for initiation of cyclic ovarian activity following parturition. Also information on the factors which influence the rate of return of the uterus to the nongravid size and function are important for determining the time of successful breeding.

In Swamp buffaloes mean values of 0.34±0.36 and 0.43±0.56 ng/ml were reported during 10 days before and after parturition. On the contrary, a progressive increase of basal LH concentration occurred from Days 2 through 35 postpartum. Mean values of 0.5±0.01, 1.3±0.03 and 2.2±0.1 ng/ml were observed for Days 2, 20 and 35 postpartum, respectively. Basal plasma LH concentration during the second and third week was inversely related to the first postpartum ovulation interval. The levels were also significantly higher in buffaloes showing estrus than in anestrous animals. Daily blood sampling from anestrous buffaloes 6–8 months after calving revealed small increases (3–9 ng/ml) probably implying estrogen secretion due to follicular growth and regression.

The levels were higher in milked than in suckled buffaloes. Fluctuations of total estrogens between 38±10 and 61±5 pg/ml (level during estrus 63±10 pg/ml) during the first 75 days postpartum in acyclic buffaloes probably reflects waves of follicular growth and atresia. As regards estrone sulphate, peak values of 7±4 ng/ml; 7±0.4 ng/ml and 6±0.1 ng/ml in the last 30–15 days prepartum were reported. An abrupt drop on the day of calving or the day before was described with basal values of less than 0.1 ng/ml reached 1–2 days postpartum. 3.2. Progesterone The literature on plasma progesterone concentrations in the last stages of gestation is rather conflicting. A clear increase of progesterone concentration was reported during the last 30–15 days. Peak values of 3±0.3 ng/ml on Day -1 for buffalo heifers and 5 ng/mL on Day -5 for mature buffaloes were noted. On the contrary gradual decrease starting 30–17 days before calving with a sharp decline as early as 8 days or as late as 1–3 days before parturition were described. Nevertheless, irrespective of the above mentioned debate, in all cases a precipitous decline of progesterone level occurred on the day of calving. In some reports basal values of 0.1–0.6 ng/ml. were reached during calving suggesting complete luteolysis at parturition, with no significant changes during the postpartum period. In others decline of progesterone continued during the postpartum period to reach minimum levels on Day 6, indicating complete regression of the corpus luteum of pregnancy. However, a wider range between 3 and 29 days for complete regression of the corpus luteum of pregnancy was reported. Demise of the corpus luteum after calving expressed by progesterone concentration on Day 3 postpartum was not different in milked and suckled buffaloes. The relationship between the patterns of estrogens and progesterone in the last stages of gestation and during calving in relation to placental delivery or retention, uterine torsion and degree of cervical dilation as well as response to exogenous glucocorticoids for induction of parturition need further studies. For a variable period after parturition progesterone levels remained basal but a transient elevation may occur before resumption of cyclic activity.

Resumption of postpartum ovarian activity indicated by follicular growth at 35–42 days; onset of luteal activity at 43–57 days or expression of estrus at <45 days are concomitant with peripheral rise of T3, T4 and BPI. Prolonged anestrus was associated with minor fluctuations of T3 and T4 levels.

10.4.2. Pathology of the postpartum period

10.4.2.1. Uterine Prolapse

Uterine prolapse is a non-hereditary complication occurring immediately after parturition and occasionally up to several hours afterwards. Prolapse of the uterus is a common complication of the third stage of labour in the cow. In ruminants the prolapse is generally a complete inversion of the gravid cornua. It has been estimated that 0.3 % to 0.5% of all calving terminate in a prolapse of the uterus. The present paper deals with a case of post-partum uterine prolapse along with fetal membranes in a non-descript buffalo. The usual sequel of uterine prolapse is haemorrhage, shock, septic metritis, peritonitis, infertility or death. Sometimes in delayed cases, partial contraction of cervix interferes with proper repositioning, resulting in recurrence of prolapse. But in this case after detaching the fetal membranes the prolapsed mass became lighter and less voluminous, so it was easy to reposite. Moreover a truss is applied, so even in the presence of a tenesmus the recurrence had not been noticed. Uterine prolapse is predisposed to a violent tenesmus and retention of fetal membrane in this case as reported. There was a drastic decrease in serum calcium (6.42 ± 1.05 v 10.96 ± 0.95 mg/dl), phosphorus (2.90 ± 0.85 v 5.50 ± 1.61 mg/dl) and magnesium (1.50 ± 0.53 v 2.40 ± 0.53 mg/dl) levels in prolapsed animals as compared to the controls. There was also a significant decrease in PCV, Hb concentration, lymphocytes and monocytes, while an increase in WBC counts and neutrophils was observed in prolapsed animals as compared to controls. However, there was no difference in haematological and serum macro mineral contents between vaginal prolapsed and uterine prolapsed buffaloes.

10.4.2.2. Metritis

The most predisposing factor for postpartum uterine infection is retained placenta and toxic puerperal metritis. The most prevalent bacteria in uterine lumen are *Escherichia coli, Archanobacterium pyogenes*, *Bacteroides fragilis* and *Fusobacterium necrophorum A. pyogenes* and *F. necrophorum* are an important pathogens causing severe uterine inflammation as found in histopathological examinations. Buffaloes with postpartum metritis showed good clinical cure when oxytetracycline injected systemically with PGF2α. Intrauterine infusion of oxytetracycline had no advantage for the treatment of uterine infection in buffalo cows with postpartum metritis. PGF2α improved clinical cure of buffaloes with postpartum metritis.

10.4.2.3. Retained placenta

The incidence of RFM in buffaloes is about 3.3%. RFM is associated with stressful condition in buffaloes and the condition lead to high incidence of infertility and culling, especially when it was associated with uterine fibrosis.

10.5. Postpartum Period in Ewes and Does

10.5.1. Physiology of the postpartum period

The postpartum fertility in sheep depends on two main factors, the involution of the uterus and the onset of postpartum ovarian cyclicity. The involuting uterus has thus been considered to be a temporary barrier to delay fertility during the early postpartum period in sheep. Nevertheless, there is no consistent effect of the many environmental factors on the rate of uterine involution. In contrast, the resumption of postpartum ovarian cyclicity in sheep has been shown to be influenced by diet and can be advanced by hormonal administration. Similarly, bodyweight losses have been found to be one of the major limiting factors of the ovarian activity resumption after parturition during the dry season while suckling as such slightly postponed the resumption of the first postpartum estrus.

Uterine involution in Ossimi ewes (fat-tail breed) was completed within two to three weeks after birth, while ovarian activity started about six weeks after parturition. The overall interval for complete uterine involution in Farafra ewes averaged 31.9±1.2 d. The diameter decreased rapidly between day 7 and day 14 postpartum (>50%), but more steadily from day 14 to day 32 postpartum. The time for uterine involution was shorter for ewes that lambed in February (end of winter) than for those lambing in June (onset of summer; 29.4±1.2 d vs. 33.9±1.1 d). The proportion of Farafra ewes (fat-tail tropical breed) that recorded luteal activity within 35 d, >35–42 d, and >42 d postpartum were 12.1%, 24.2%, and 63.7% for the February lambing; and 53.7%, 36.6%, and 9.7% following the June lambing, respectively. No effect was recorded regarding the parity, the litter size, and dam body weight at parturition, or their interactions on the interval to the onset of the postpartum luteal activity. Ewes that lambed in June (summer) recorded a serum progesterone concentration of more than 1 ngml−1 earlier than for those that lambed in February (winter; 39.0±1.2 d vs. 69.3±1.2 d, respectively). Moreover, following the June lambing three of the five ewes exhibited a complete estrous cycle (17–18 d in length), and two of the five ewes displayed a short estrous cycle (7–9 din length) within the observation period. In contrast, following the February lambing season, none of the ewes exhibited a complete estrous cycle (0/6), and only one ewe (1/6) showed a short cycle (11 d in length) within the same period. No relationship was recorded between the onset of the luteal function and the interval to complete uterine involution.

10.5.2. Pathology of the postpartum period

10.5.2.1. Uterine Bacterial contamination

In postpartum period, the uterus seems to be able to prevent bacteria from achieving infection unless suppression to uterine defense mechanism occurs. *Trueperella pyogens*, *Escherichia coli* and *Streptococci* Spp. are commonly isolated from uteri of ewes. Sheep postpartum endometritis, postpartum acute inflammation of the endometrium, often occurs after delivery because of dystocia, placenta retention, uterine prolapse, and incomplete uterine involution. Symptoms includes depression, fever, loss of appetite or waste must, ruminant reduced or stopped, mild drum gas, Arch, dedicated efforts, sticky discharge from the vulva or mucopurulent secretions, secretions were serious dark red or brown, and smell, especially when lying under the discharge more. Without timely treatment, or

treated properly, can be transformed into chronic, often secondary to pyometra. Treatment for the elimination of inflammation includes application of ampicillin sodium. Tetracycline, or oral application of sulfamethoxazole can be used. In order to promote uterine contraction and enhance the defense function of the uterus to remove the uterus cavity exudates, can be used oxytocin (10 to 50 international units). To improve the general situation 10% calcium gluconate injection intravenously, each with 50 to 150 ml can also be used.

10.5.2.2. Uterine prolapse, retained placenta and metritis

In previous studies, Awassi ewes with uterine prolapse were treated by repositioning and suturing of the vulva for 3–5 days. Good prognoses were obtained in 80% of the cases. Animals affected with retained placenta were treated by manual removal of placenta plus long acting (LA) oxytetracycline 20% i.m. ($n = 37$) or 2 mg oestradiol benzoate i.m. plus 20 IU oxytocin i.m. ($n = 37$) or 10 mg prostaglandin F2α i.m. ($n = 36$). Good response was obtained (91.6%) with PG F2α treatment. Manual treatment, antibiotics and oestradiol benzoate plus oxytocin had a response of 54.8% and 67.5%, respectively. There was a difference ($P < 0.01$) between prostaglandin-treated and other groups. Ewes with postpartum metritis were treated: Group 1 ($n = 25$) 20 mg per kg body weight (BW) L.A. oxytetracycline i.m.; Group 2 ($n = 250$ 20 mg kg−1 BW L.A. oxytetracycline i.m. plus oestradiol benzoate (2mg i.m.) followed by 20 IU of oxytocin i.m.; Group 3 ($n = 24$) 10 mg prostaglandin F2α i.m. plus 20 mg kg−1 BW L.A. oxytetracycline i.m. High success was obtained (91.6%) in the third group, while the responses were 60% and 76% in the first and second groups, respectively, with a difference ($P < 0.01$) between groups. It was concluded that Caesarean section was the most successful procedure for treatment of dystocia. It was also documented that injection of PG F2α or oxytocin plus oestradiol alone or in combination with antibiotics directly after parturition reduced morbidity from retained placenta and postpartum metritis.

Suggested Readings

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APPENDIX:Tables and Figures

Table 22: Characteristics of the postpartum period in farm animals

	Female camels	mares	cows	Buffalo-cows	Ewes, does
Time for uterine involution	18-30 days	9-32 days	25-45 days	20-35 days	20-25 days
Time for regeneration of the endometrium	10-14 days	10-14 days	25 days	25 days	28 days
Time for onset of the ovarian activity	20-30 days	5-9 days	20 days	35 days	14-21 days
Time to first estrus	15-50 days	5-12 days	30-60 days	45-90 days	25-40 days
Lochia	4-7 days: reddish	24-48h: reddish, little	2-3 days: reddish, large	Like cattle	Like cattle, but
	After d 7: scanty, yellowish, viscous	amount, less viscous	amount 6-8 days: chocolate, fair amount 14-18: whitish, scanty Amount: primipara: 50 ml; Pluripara: 800-2000 ml		lesser amount

Fig. 71: Uterine prolapse in female camels (*Qassim-KSA, 2007-2011*).

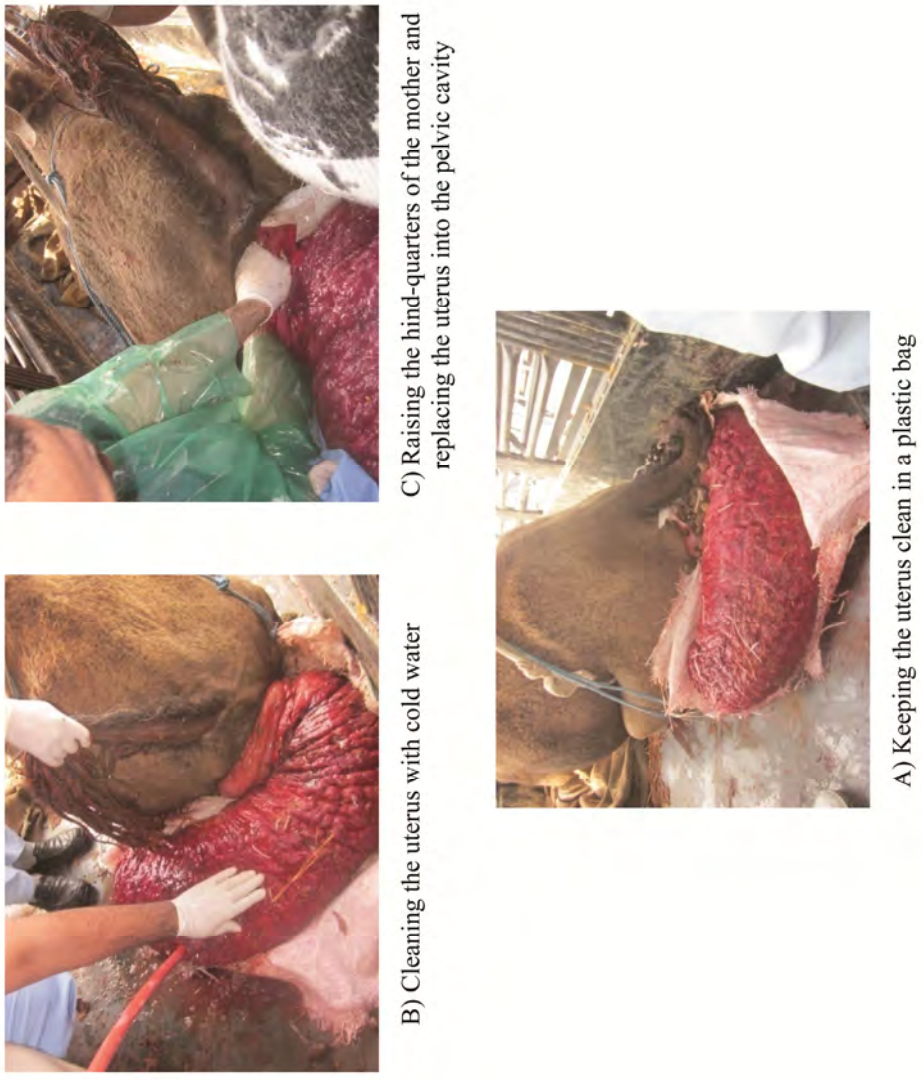
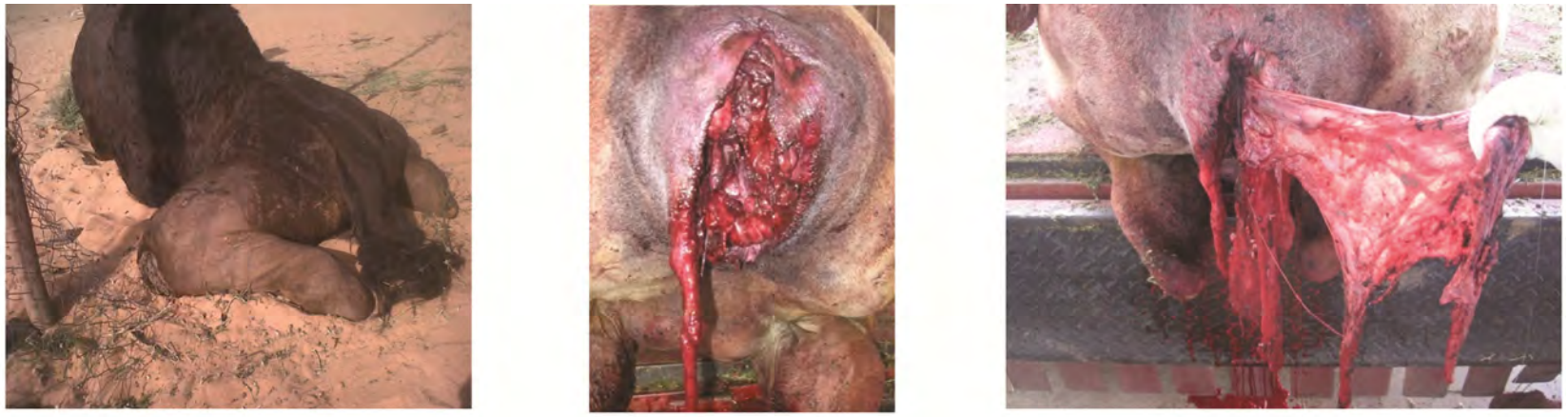


Fig. 72: Treatment of uterine prolapse in female camels (*Qassim-KSA, 2010*).



A) Sternal recumbence due to parturient paralysis

B) Tearing of vagina and vulva

C) Retained placenta

Fig. 73: Postpartum complications in female camels (*Qassim-KSA, 2007-2012*).

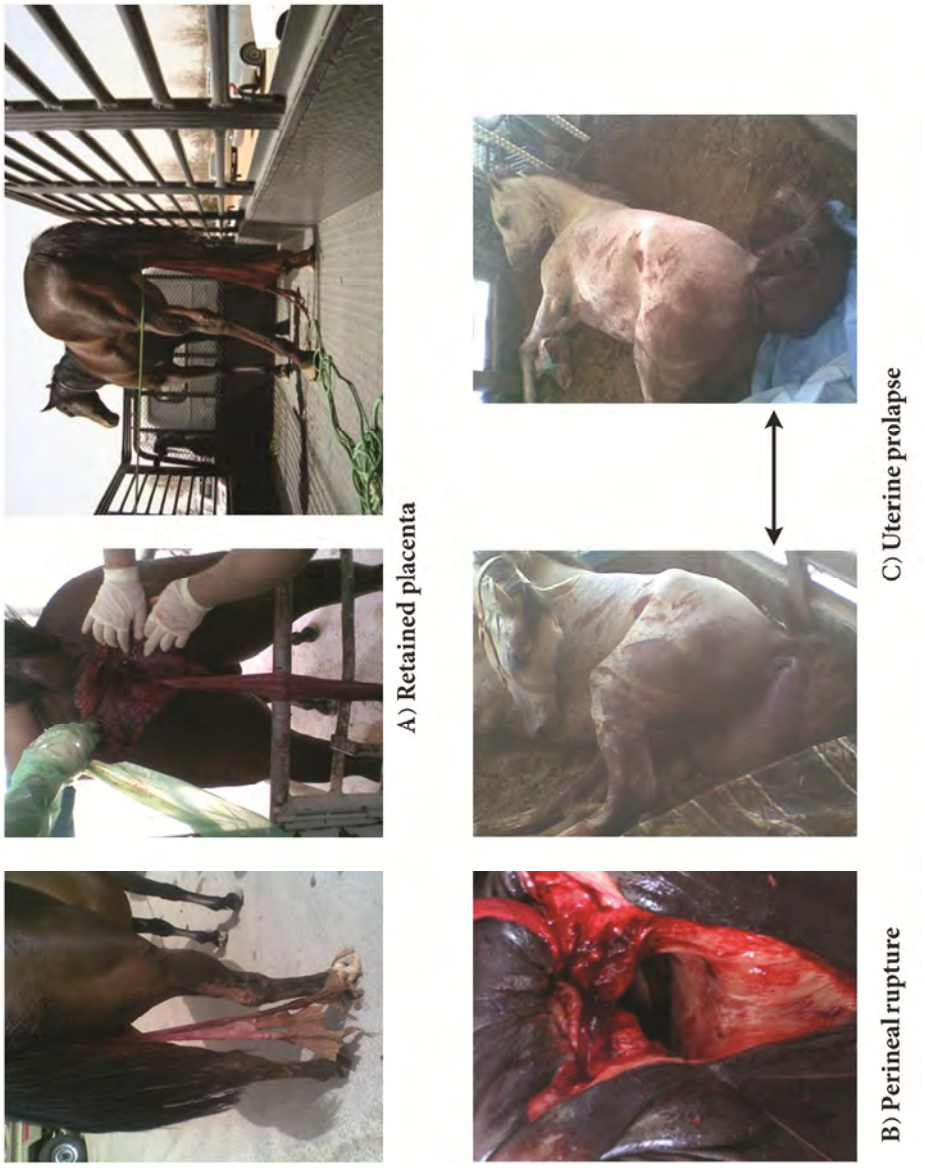
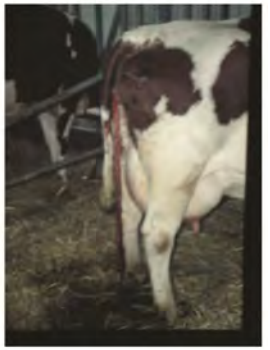


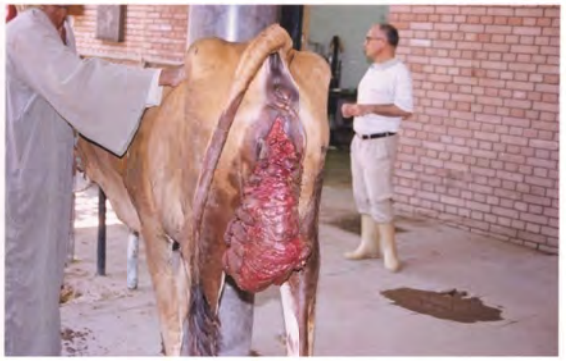
Fig. 74: Postpartum complications in mares (*Qassim-KSA, 2007-2013*).

A) Sternal recumbence due to injury of the obturator nerve

B) Necrotic vaginitis



C) Retained placenta

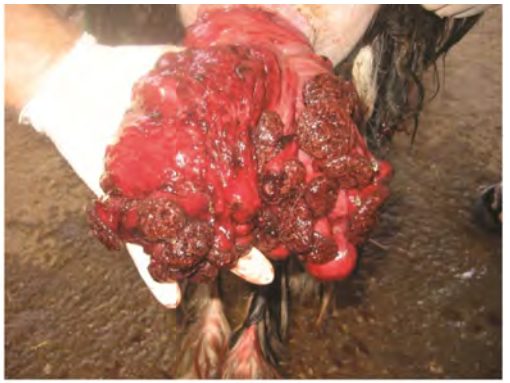


D) Uterine prolapse

Fig. 75: Postpartum complications in cows (*Berlin-Germany, 1998; Assiut-Egypt, 2003; Qassim-KSA, 2010*).

A) Uterine prolapse - goat

B) Complete uterine and vaginal prolapse - goat



C) Uterine prolapse - goat

Fig. 76: Postpartum complications in goats (*Qassim-KSA, 2007-2013*).

Chapter 11

Male Fertility and Infertility

The reproductive organs of the male consist of the testicles, secondary sex organs, and the accessory sex glands. These organs work in concert for formation, maturation and transport of spermatozoa, which are eventually deposited in the female reproductive organs. The secondary sex organs are the **epididymis, vas deferens** and **penis.** The accessory sex glands include the **seminal vesicles, prostate** and **bulbourethral gland** (Cowper's gland).

The testicle is located outside the body cavity in the scrotum and has two vital functions: producing the spermatozoa, and producing the male hormone, testosterone. Location of the testicles exterior to the body cavity is essential for normal sperm formation, which occurs only at 4 to 5 degrees below body temperature. The scrotum provides physical protection to the testicle and helps regulate the temperature for optimum spermatozoa development. This regulation is done by coordination of three structures: a temperature-sensitive layer of muscle (tunica dartos) located in the walls of the scrotum, which relaxes when hot and contracts when cold; the external cremaster muscle within the spermatic cord, which controls the proximity of the testicle to the body by lengthening or shortening depending on environmental temperature; and a counter-current temperature exchange regulated by a blood flow process known as the *pampiniform plexus,* which is a coil of testicular veins that provide an effective mechanism for cooling arterial blood entering the testicle and transferring its heat to the venous blood leaving the testicle. The testicle contains many long, tiny, coiled tubes known as *seminiferous tubules,* within which the sperms are formed and begin to mature. Scattered throughout the loose connective tissue surrounding the seminiferous tubules are many highly specialized cells, the *interstitial cells of Leydig,* which produce testosterone. There are hundreds of individual seminiferous tubules in the body of the testicle which unite with one another to form a few dozen tubules that exit from the testicle and pass into the *epididymis*.

The epididymis is a compact, flat, elongated structure closely attached to one side of the testicle. It is divided into three regions, the head, body and tail. The many tubules entered the head of the epididymis from the testicle unite to form a single tubule. This tubule is convoluted and packed into the 6- to 8-inch epididymis. Four major functions occur in the epididymis, including the transport of the developing sperm cells from the testicle to the *vas deferens*; the concentration of the sperm by absorption of surplus fluids; the maturation of the developing spermatozoa; and the storage of viable sperm cells in the epididymal tail. If sexual activity is slowed, resorption of sperm cells from the epididymal tail occurs. The epididymis serves as an outlet for all the sperm produced in the testicle and any blockage of this tube will cause sterility.

The vas deferens, also known as ductus deferens, emerges from the tail of the epididymis as a straight tubule and passes as part of the spermatic cord through the *inguinal ring* into the body cavity. Spermatozoa are transported further along the reproductive organs to the pelvic region through the vas deferens by contraction of the smooth muscle tissue surrounding this tubule during ejaculation. Bulls may also be sterilized by a vasectomy in which a section of the vas deferens is removed so that sperm cannot pass to the outside of the body.

The two vas deferens eventually unite into a single tube, the urethra, which is the channel passing through the penis. The urethra in the male serves as a common passageway for semen from the reproductive organs and urine from the urinary organs.

Two of the accessory glands are found in the general region where the vas deferens unites to become the urethra. Secretions from these glands make up most of the liquid portion of the semen. In addition, the secretions activate the sperm to become motile. The seminal vesicles consist of two lobes about 4 to 5 inches long, each connected to the urethra by a duct. The *prostate gland* is located at the neck of the urinary bladder where it empties into the urethra. The prostate is relatively small in the bull, as compared to other species, and does not produce a very large volume secretion.

The third accessory gland, the *Cowper's glands* are small, firm glands located on either side of the urethra. The clear secretion that often drips from the penis during sexual excitement prior to service is largely produced by these glands and serves to flush and cleanse the urethra of any urine residue that may be harmful to spermatozoa.

The *sigmoid flexure* is an anatomical structure that provides a means by which the penis is held inside the sheath except during time of service. Strong retractor muscles hold the penis in the "S" shaped configuration. Occasionally these muscles are too weak to function properly and a portion of the penis and sheath lining protrude at all times. This exposes the male to the danger of injury and this characteristic should be avoided when selecting a herd bull.

The *penis* is the organ of insemination. Spongy-type material within the penis is filled with blood during sexual arousal, resulting in erection of the organ. The end of the penis is the glans penis and is richly supplied nerves, which are stimulated during copulation to induce ejaculation. Impairments of the glans penis may exist and should be detected during a fertility exam

Regulation of male hormones

The testicle functions as an endocrine gland because of its production of the male hormone, **testosterone,** by the interstitial cells. Testosterone has several major functions: it is largely responsible for development and maintenance of the male reproductive organs. It causes the development and maintenance of the secondary sex characteristics associated with masculinity, such as the crest and heavily muscled shoulders. It is a major factor in the normal sex drive and behavior of the male. It increases muscular and skeletal growth. It is essential for normal sperm formation. LH and FSH are released from the pituitary gland and cause the testicle to secrete testosterone, which then acts on the germ cell lining of the seminiferous tubules to stimulate formation of primordial sperm cells. The maturation of spermatids into fully developed sperm cells requires the presence of FSH. The level of

testosterone in the blood regulates the secretion of gonadotropic hormones from the anterior pituitary via a feedback system. A proper balance of all hormones is vital to successful reproductive functions.

Breeding soundness examination

An examination of male for breeding soundness before the breeding season can detect the majority of males which have obvious potential fertility problems. Guidelines for a breeding soundness examination include: The penis should be examined during electro-ejaculation or natural mating, in an erected, extended state. Potential problems of the penis may include hair rings, which restrict circulation, a persistent frenulum or adhesion, lacerations, growths, scar tissue, deviations, or a urethral fistula. Next the scrotum and testes should be palpated. Except in male camel, the scrotum should be pendulous but well supported. The testes should be firm and uniform in size and shape. The internal sex organs should be palpated rectally to ensure proper development and size. Good vision and sound feet and legs should also be considered when evaluating a bull's physical abilities to breed.

Scrotal circumference gives an indication of the ability to produce sperm. Evaluation of semen characters includes: volume, concentration, motility and morphology.

Breeding soundness examinations include an evaluation of a bull's sexual drive or libido. Libido testing requires use of females in estrus and therefore. Procedures are being investigated to better evaluate the willingness and desire of males to mate.

Semen and spermatozoa

Semen consists of spermatozoa suspended in seminal plasma. The latter is derived from multiple sources, including the testes, epididymides, and accessory glands. Seminal plasma contains sperm metabolites (including fructose, citric acid, sorbitol, glycerylphosphorylcholine and inositol), amino acids, enzymes, antimicrobials, hormones, and immunoglobulins. Spermatozoa consist of a flattened head, midpiece, and tail (with principal and end pieces). The sperm head is a specialized package of condensed chromatin containing DNA, surrounded by a nuclear membrane, with an acrosome on its anterior aspect. The presence of an intact acrosome is an essential prerequisite for fertilization as it is the site of capacitation and the acrosome reaction.

Sperm transport and ejaculation

Immotile spermatozoa are carried into the lumen of the seminiferous tubule following separation of their connections to Sertoli cells within the spermatogenic epithelium. Most of the residual cytoplasm is retained by the Sertoli cell, although some remains attached to the spermatozoa (as cytoplasmic droplets). Initial transport into the rete testis seems to be dependent upon fluid secreted by Sertoli cells. Once into the efferent ducts, sperm movement is facilitated by ciliated epithelial cells (within the ducts) as well as by smooth muscle contractions. The efferent ducts and initial segment of the caput epididymidis resorb most fluid and protein emanating from the testis and secrete new compounds. Transportation of spermatozoa within the epididymis is primarily due to smooth muscle contractions. As spermatozoa progress through the epididymis, they attain the ability to be progressively motile, the cytoplasmic droplet migrates from the proximal to the distal position on

the sperm midpiece, and fluids are resorbed and exchanged. Through these and other changes, the epididymis plays a pivotal role in preparing sperm for fertilization. The availability of testosterone, particularly in the initial and middle segments of the epididymis, is an important factor in sperm maturation. The epididymis, particularly the tail region, acts as a storage region for sperms. Most sperms that are not ejaculated are voided in urine, with a small proportion resorbed by the male tract. Sperms are transported from the cauda epididymidis to the urethra in the ductus deferens (vas deferens) via muscle contractions that are strongest during precoital stimulation. The terminal portions of the ductus deferens expand to form the ampullae; these act as minor sperm storage areas as well as secreting fructose and citric acid into the seminal plasma. The ductus deferens opens (via the ampullae) into the cranial portion of the pelvic urethra. The vesicular glands (seminal vesicles) also open into the pelvic urethra, providing much of the fluid component of the bull ejaculate, as well as sperm nutrients and semen buffers. These lobulated glands are approximately 10 to 15cm in length and 2 to 4cm in diameter in mature bulls. The prostate gland, consisting of a relatively small body and larger disseminate region, produces 25% to 40% of seminal volume as well as semen odor. The bulbourethral glands each open into the pelvic urethra at the ischial arch. The urethra is an elongated tube extending from the bladder to the tip of the penis. It is surrounded by the urethralis muscle, which contracts strongly during ejaculation. The penis of the bull is fibroelastic with erection causing relatively little increase in penile diameter. Retraction of the penis into the sheath is controlled by the retractor penis muscle. Ejaculation coincides with the penis reaching its maximal length and momentary peak pressure within the corpus cavernosum penis (CCP) as high as 14,000mmHg; the pressure is due to vascular engorgement of the CCP caused by rhythmic contractions of the ischiocavernosum muscle, forcing blood anterior from both crura.

Comparative anatomy of the genital organs indifferent male animals is shown in Table (23). Morphology and pathology of the male genital tract in each animal species are illustrated in Figs. (77-96).

11.1. Male Camels

11.1.1. Anatomy and physiology of the reproductive organs

The testes of *camelidae* are ovoid in shape and are found in the scrotum in a perineal position. The testes of camels are usually descended at birth but are very small. They increase in size at the onset of puberty and vary in length from 7 - 10 cm and weigh between 80 - 100 g each. They become enlarged and protrude when the male camels are sexually active in the rutting season. The epididymis is located at the anterior edge of the testis and extends from the interior extremity to just above the upper edge.

The vas deferens is enclosed in the spermatic cord and measures 45 - 50cm in camels.

The penis of *camelidae* is of the fibroelastic type and relies primarily on its elasticity for erection and extension. In the absence of an erection, the penis is retracted into its sheath via a prescrotal sigmoid flexure not a post scrotal sigmoid flexure, as is the case in bulls. The glans penis is 8 - 12 cm long and ends in a cartilaginous process which supposedly directs the penis through the cervix of the female during copulation.

The most important feature in the anatomy of the internal genitalia of the *camelidae* is the absence of seminal vesicles. In the dromedary, the accessory sex glands are the ampullae, the prostate, the bulbourethral (Cowper's), and the urethral glands.

In a study of the authors using caliper and ultrasonography, the three dimensions of the testes and epididymal tail and head were found to increase with the advancement of camel age. The left testes measurements were bigger than the right but not statistically significant. There were significant differences between the BUG measurements in age groups. The differences between pars disseminata of the prostate gland and pelvic urethra in the pre-pubertal and sexual mature group were significantly different. The obtained data could provide a useful tool for predicting camel puberty and future fertility.

11.1.2. Puberty and Sexual Maturity

Puberty in the male is defined as the time when he is capable of the mating act and getting the female camels pregnant. This definition usually assumes the presence of normal sexual behaviour and spermatogenesis. In dromedary camels sexual behaviour has been observed as early as 2 years of age, but most field observations suggest that puberty and fertilizing ability are not reached until 3 to 5 years of age. In traditionally managed dromedary herds the males are not used for breeding before the age of five and normal sexual activity continues until 20 years of age, when some males begin to show signs of senility changes in their sexual behaviour or sperm production.

For dromedaries, the breeding season is very variable but coincides in general with the period of low environmental temperature, low humidity and increased rainfall (winter season). The length of the breeding season depends both on climatic and nutritional parameters. The onset and duration of the rutting season are also affected by the type of management and the individual male. Males that are loose in a herd of females tend to come into season earlier and remain in rut for a longer period than confined males.

11.1.3. Sexual behavior

The onset of the breeding season is characterized by the display of a series of behavioral changes called the "rut". Aggressiveness - with the onset of the breeding season the male dromedary gets increasingly aggressiveness towards other animals in the herd (especially other males) and sometimes even towards people. They become easily excitable and often very hard to handle. Confined males show increased pacing and anxiety and may make several attempts to break out of the corral or pen. During the breeding season, male dromedaries spend most of their time guarding the herd and surveying it for the presence of receptive females or any approach by strange males. Because of this continuous stress, a net reduction of food intake and frequent diarrhea all males tend to lose

weight (up to 35% of their original bodyweight), sometimes to the point of emaciation. Sexual behavior in the camel is also characterized by exteriorization of the soft palate, known as the Dulah. The protrusion of this structure occurs all day and becomes more frequent as the male gets increasingly excitated ie. by the presence of another male or if females pass nearby. The male also frequently produces a metallic sound by grinding the molars with lateral movements of the lower jaw. This sound can be produced at any time, but usually replaces the gurgling and soft palate ejection during copulation. Marking is one of the main sexual behaviors exhibited during the rutting season for the male to define his territory and it usually takes two forms: urine spraying and poll-gland secretions smudging. During the spraying activity, the male dromedary assumes the crouched urination posture, while his hind quarters are flexed slightly with legs widely spread. Urine is ejected backwards in small quantities and spread over the croup of the animal and surrounding areas with regular tail beating. During the breeding season, the poll gland secretion is very copious and becomes black and tarry in consistency. It dribbles down the neck of the sexually active male and has a very strong, fetid smell. These secretions probably contain androgens and some type of pheromones that are used to mark a territory. The rutting dromedary displays a frequent rolling and rubbing activity of the neck on small bushes or on the sand, especially when he is introduced into a new environment. During the breeding season, male dromedaries will continuously seek receptive females. They sniff the flank and perineum of females and frequently display a flehmen reaction i.e., curling up of the top lip. Males which are running free in the herd will often chase individual females and force them down for breeding even if they are not receptive. This could be an important factor in the weight loss of the male dromedary observed during the breeding season.

Mating is carried out with the female sitting in sternal recumbence and the male squatting behind her with his hind legs completely flexed and his forelegs extended on either side of the female. The duration of copulation is very variable and can be affected by breed, age, season and frequency of use. It tends to decrease as the weather becomes warmer and at the end of the breeding season. On average, dromedaries mate for 5 minutes, but this can range from anything between 3 - 25 minutes.

11.1.4. Breeding soundness evaluation

An important part of this evaluation is history, which can only be obtained if the breeder has kept detailed records on the animal in question to address the important factors affecting reproduction. A complete general physical examination should be performed to determine the overall health of the animal as well as the absence or presence of defects that compromise the breeding potential. If the examination is conducted to select a young herd sire, an emphasis should be put on detection of congenital and potentially heritable conditions. Examination of the reproductive organs focuses on palpation of the scrotum, testes and prepuce to detect any signs of inflammation or traumatic lesions. The scrotal and preputial skin is often the site laceration, insect bite or parasitic lesions (i.e. mange). Increase in scrotal skin thickness may result in loss of cooling ability and deterioration of spermatogenesis. The testes should move freely within the scrotum, be nearly equal in size and resilient to palpation and non-painful. The preputial orifice should be checked for discharge, obstruction and prolapse. Testicular size is a very important indicator of sperm production ability and

fertility. Size of the testis is evaluated by measuring its width and thickness. Difference in testicular length between left and right testis should be less than 15%.

An ultrasound examination of the scrotum and its contents cannot be overemphasized as many testicular abnormalities cannot be felt on palpation. The normal scan should show a homogenous testicular tissue with a more echogenic center (mediastinum testis). Abnormalities detected using this technique include testicular or epididymal cysts, accumulation of fluid around the testicle (hydrocele) or presence of fibrous lesions (scar tissue).

Mating ability of the male is best evaluated in the presence of a receptive female. Physical mating ability and sexual behavior can be assessed during the courting and copulation phases of mating. The penis may be examined in the erect state at the time of intromission. Detailed examination of the prepuce and penis may require restraint, sedation or even anesthesia of the animal.

Breeding soundness evaluation should be completed by determination of at least motility and morphology of a semen sample taken after mating as described above.

For some specific conditions (unexplained infertility or subfertility, testicular asymmetry, and abnormal testicular ultrasonography); it may be necessary to take a sample of testicular tissue by either biopsy or needle aspirate. This technique is useful for diagnosis of reasons for azoospermia and testicular cancer. Endocrinological evaluation of males with poor reproductive performance is still not well developed in camelids.

11.1.5. Reproductive disorders

In one study for the authors, a total of 32 male dromedary camels were examined due to post-coital infertility (impotentia generandi). They were between 6 and 18 years of age. The breeding soundness examination began with a thorough history. The males were then restrained in a sitting recumbence and a general physical examination, the testes and epididymides were evaluated for size, shape, and consistency using visual inspection, caliper and ultrasound. Semen was collected by electroejaculator. The ejaculate was analyzed for color, volume, sperm concentration, individual motility, viability and morphology. Testicular biopsy was obtained for histopathology. Of the affected male camels, apparently normal testicles, unilateral or bilateral testicular aplasia, unilateral or bilateral testicular hypoplasia, and unilateral or bilateral testicular enlargement have been observed. The other parts of genital organs were apparently normal in all animals. All observed male camels had one or more defect in their seminal traits. The volume of the ejaculate was within the normal range in most cases. The sperm cell concentration was 0.0 sperm/mm3 (Azoospermia) in 35% of cases, between 10000 and 25000sperm/mm3 (oligospermia) in 40% of cases, and > 400000sperm/mm3 in 25% of cases. The vitality of spermatozoa was between 0.0% and 50% in 63% of cases and > 50% in 27% of cases. The sperm cell abnormality was > 50% in 60% of cases and < 50% in 40% of cases. Maturation arresting, hypospermatogensis, necrosis and desquamation of germ cells, seminoma like structures, and atrophy and firbrosis of semineferous tubules were observed in the testicular biopsy of the affected male camels.

In a further study, 25 male dromedary camels were examined for inability to copulate (impotentia counendi). They were between 5 and 15 years old. Andrological examination revealed defects in the prepuce in 40% of cases (edema and narrowing or adhesion), defects in the penis in 35% of cases (flaccid penis, balanitis, injured penis), and defects in scrotum or testicles in 10% of cases (odema, enlargement). The genital organs of the rest of cases (15%) were apparently normal.

11.2. Stallions

11.2.1. Anatomy and physiology of the reproductive organs

The anatomic features involved in the reproductive physiology of stallions include the pineal gland, hypothalamus, pituitary gland, and testis. The pineal gland is located between the cerebral hemispheres and lies dorsal to the pituitary gland. This gland produces the hormone melatonin in response to visual light signals it receives from the retina. In long-day breeders such as horses, an increase in duration of light exposure results in a decrease in melatonin production by the pineal gland. Melatonin is released in the greatest quantities during the hours of darkness. High levels of melatonin have the effect of lowering levels of gonadotropin-releasing hormone (GnRH), possibly through an influence of the feedback mechanisms exerted by androgens on GnRH release. Located at the base of the brain, the hypothalamus produces GnRH in a pulsatile manner in response to a variety of stimuli. Olfactory, tactile, auditory, and visual signals alter the production of GnRH by the hypothalamus, which is transported via portal vessels to the anterior pituitary gland. Pulsatile secretion of GnRH stimulates the production and pulsatile release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the pituitary. Within the testis, the seminiferous tubules of the adult stallion make up about 70% of the testicular parenchyma. The tubules consist of Sertoli cells and germ cells in various stages of development. The Sertoli cell is both a structurally and physiologically supportive cell to the process of spermatogenesis. Developing sperm cells are embedded into, or in intimate contact with, the Sertoli cells throughout maturation. Most of the remainder of the testis consists of Leydig cells, myoid cells, and blood vessels of the interstitial compartment. In adult stallions, pituitary LH binds to Leydig cell receptors, stimulating the production and release of estrogens and testosterone by the cells. Pituitary FSH stimulates the production and release of multiple factors from the Sertoli cells of the testis, including inhibin, activin, androgen-binding protein (ABP) and insulin-like growth factor (IGF). These testicular hormones and proteins feed back to the hypothalamus to regulate the pulsatile release of GnRH and therefore the continued production of LH and FSH. Estrogens, not testosterone, produced by the Leydig cells appear to regulate LH release from the pituitary gland of the stallion, either at the level of the hypothalamus by altering GnRH pulse amplitude and frequency, or directly at the level of the pituitary.

11.2.2. Puberty and sexual maturity

Although most stallions begin to produce sperm as early as 12 to 14 months, most are at least 15 months or older before they can successfully breed. Few stallions are used at stud before two years of age and most stallions acquire full reproductive capacity at around three years of age.

11.2.3. Sexual behavior

A stallion with a high libido will exhibit an eagerness to mount and attempt to breed a mare. In natural situations, stallions exhibit a wide range of libido levels, from zero activity to extreme aggressiveness. Some stallions will have such a strong libido that they will sacrifice all other pursuits in favor of searching for and breeding mares in heat. An extremely high or low libido may cause problems. Young stallions are more likely to exhibit a wide range of libido. Young stallions with extremely low libido are hard to breed and require patience from those handling them. Young horses with very high libido require extreme caution by the handler and those working the breeding shed.

11.2.4. Breeding Soundness Evaluation

The stallion breeding soundness evaluation is intended to *estimate* a stallion's reproductive potential, which is the current ability of a stallion to impregnate mares, resulting in the birth of a normal foal. This *estimation* includes breeding history, physical examination of the whole stallion, physical examination of the reproductive organs, breeding behavior, determination of bacterial growth in association with the reproductive organs, and semen quality measurements. Following the description and listing of the foregoing characteristics, the clinician categorizes the stallion into one of three classifications: Satisfactory, Questionable, and Unsatisfactory. If a stallion is considered deficient in two or more of these categories (total scrotal width, progressively motility, percent morphologically normal sperm), he is considered either a Questionable or Unsatisfactory prospective breeder. The difference between these two categories is a matter of degree.

11.2.5 Reproductive disorders

Penile trauma may be caused by the mare or from events associated with breeding. When a mare kicks a stallion during breeding, there may be damage to his external genitalia, resulting in swelling, edema, abrasions, or lacerations. Blunt trauma to the penis and sheath may cause enough swelling to prevent retraction of the penis into the sheath (paraphimosis). With the penis swinging free outside the sheath, normal blood circulation to the glans is inhibited, leading to further swelling and edema. The initial treatment consideration in the case of trauma should be stall confinement to prevent further damage. Second, the penis should be replaced into the stallion's sheath. With minimal swelling, the penis may be easy to replace. When edema is extensive, the swelling should be reduced before replacement within the sheath is attempted. Application of a compression bandage beginning at the glans penis may decrease swelling sufficiently for replacement. With the penis inside the sheath, non-absorbable sutures should be placed across the sheath opening. Retaining the penis in the sheath will allow return of normal blood circulation, reduce edema, and prevent further damage. The stallion can urinate without extending the penis.

Hemospermia may result from either external or internal lesions in the stallion's reproductive organs. External lesions include those from traumatic injury such as lacerations, hematomas, abrasions, ulcerations, and dermatologic conditions. Lacerations and abrasions may occur during breeding, as when the mare's tail hairs cut the glans or urethral process during coitus. Hematomas may rupture externally or into the urethra causing spontaneous bleeding or hemorrhage during urination or

ejaculation. Diagnosis of hemospermia may begin by observation of accumulated dried or fresh blood on the stallion's rear legs, sheath, or ventral abdomen. Fresh blood may be noted on the stallion's penis following coitus or may be visible grossly in the collection container after semen collection. If fresh blood appears on the stallion's penis following coitus, the source of hemorrhage could be from the mare's genitalia (vaginal or hymen rupture, or trauma of a varicocele within the vestibule) or from the stallion. To determine the origin of blood, the mare must first be ruled out as a source, then the stallion's penis and sheath should be examined with a small-diameter flexible endoscope, with care taken to prevent iatrogenic urethral trauma. The examiner should be cognizant that ulcers may occur anywhere along the urethra. The most frequent location for urethral ulcers may be in its pelvic portion as the urethra bends distally over the ischial arch, or near the ejaculatory ducts at the cranial termination of the urethra. Treatment for hemospermia consists of sexual rest, local medications placed in the pelvic urethra via a perineal urethrostomy, systemic antimicrobial administration, acidification of the urine, or surgery (perineal urethrostomy). Laser surgery may be useful for cautery of the ulcer(s). Dermatologic Conditions Invasive dermatologic conditions, such as habronemiasis, lead to ulcers and can be found on the urethral process, glans, or prepuce. The ulcerated area can hemorrhage or cause pain during ejaculation. Recommended therapy usually consists of a combination of topical medications designed to kill the Habronema larvae and protect the mucosa, sexual rest to allow time for healing, systemic anthelmintics or glucocorticoids or both, or cautery of the lesions.

Lameness or injury may become related to fertility, especially when it involves rear limb problems, because it may delay or prevent proper mounting and copulation or lead to behavioral problems. Necessary therapy may range from surgical intervention to administration of topical or systemic medications designed to alleviate pain associated with copulation.

After recovery from the physical trauma, the stallion may exhibit abnormal breeding behavior such as refusal to mount mares, mounting without intromission, and ejaculation failure. Psychologically affected stallions should be exposed only to mares at the peak of estrus; these mares must be as receptive and gentle as possible. When a session results in ejaculation and preinjury behavior, the stallion should be properly rewarded and praised and then removed from sexual contact until the next day.

Urination during ejaculation (Urospermia) will likely cause subfertility and may be caused by psychological abnormality or organic disease. If the condition is behavioral, it may be amenable to treatment as outlined for other behavioral conditions. Management may include breeding or collecting semen only after observed urination. Administration of a diuretic followed by placing the stallion in a freshly bedded stall may assist this process.

Endocrine abnormalities or imbalances can dramatically affect fertility. Currently, measurement of follicle-stimulating hormone (FSH), luteinizing hormone (LH), total estrogens (TE), testosterone, thyroxin (T4), tri-iodothyronine (T3), cortisol, inhibin, and insulin in stallions is available. Imbalances of TE and FSH in stallions can be associated with subfertility. It has been shown that elevated concentrations of FSH and decreased concentrations of inhibin and TE are almost always associated with subfertility and onset of testicular degeneration, as evidenced by an increased number of breedings per pregnancy. Circulating concentrations of LH and testosterone should be measured

before and after challenge with exogenous gonadotropin-releasing hormone (GnRH). Stallions with idiopathic testicular dysfunction will display low motility (20–30% progressive motility), and marginal morphology (±40% normal sperm) along with some slight change in testicular consistency. Subfertile stallions may have low testicular response with respect to GnRH challenge tests because of a dysfunctional hypothalamic-pituitary axis producing bio-inactive LH, or a primary testicular dysfunction. Testicular biopsy may serve as an early indicator of endocrine/paracrine/autocrine dysfunction when used diagnostically in stallions being assessed for breeding soundness. The testicular tissues are submitted for direct endocrine analysis. The efficacy of exogenous gonadotropin for treatment of stallion infertility has been seriously questioned.

Systemic infectious diseases may decrease fertility by causing reproductive organs inflammation or through their effects on other systems. Such conditions may be readily transmissible through a variety of means, including venereal. Treatment should be aimed at rapid elimination of the primary cause of the illness and should allow resumption of normal sperm production. After a 60-day rest, an estimate of fertility based on evaluation of semen quality will help determine when the stallion may be able to return to the previous breeding level.

Subfertility caused by bacteria or viruses may affect the internal or external reproductive organs.

Many bacteria have been considered part of the normal flora. Pathogenic bacteria that should be of concern are *Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus zooepidemicus, Taylorella equigenitalis,* and, rarely, others. These bacteria may be transmitted venereally to susceptible mares or exist in the stallion without being transmitted to mares. Overt clinical signs of bacterial infection of the internal or external genitalia may not be apparent. Rather, infection is suspected based on assessment of breeding results and culture of the reproductive organs. Confirmation of suspected subfertility should begin with an indication that mares have been bred during several cycles without conception; reduced pregnancy rate; an increased incidence of early embryonic deaths; or a combination of these findings.

11.3. Bulls

11.3.1. Anatomy and physiology of the reproductive organs

The reproductive organs of the mature bull include paired testes, each with a spermatic cord, an epididymis, and a deferent duct (ductus deferens), which culminates in an ampulla. In addition are paired vesicular glands, a prostate gland, paired bulbourethral (Cowper's) glands, and a fibroelastic penis, which incorporates a sigmoid flexure. The testes are suspended within the scrotum, a feature that is important for testicular thermoregulation. Within the testis, most (70% to 90% by weight) of the parenchyma is composed of seminiferous tubules (Sertoli cells and layers of germ cells). The remainder consists of interstitial tissue (Leydig cells, blood and lymph vessels, and connective tissue). The mediastinum, an area of connective tissue extending lengthwise in mid-testis, contains blood vessels and tubules of the rete testis. As testes weight is highly correlated with scrotal circumference,

this highly repeatable measure has gained wide acceptance as an estimate of sperm-producing capability, especially as testicular size is heritable in beef bulls.

The epididymis, an elongated, torturous duct extending from the rete testis along the medioposterior border of the testis, comprises the head (caput), body (corpus), and tail (cauda) regions. Epididymal functions include sperm transport and maturation, as will be discussed. At birth, the bull penis is short and slender and lacks a sigmoid flexure, and its apex is fused to the inner lining of the prepuce. With time (and under the influence of androgens), penile and preputial tissues separate, the penis elongates, and a sigmoid flexure develops. Tissue separation proceeds irregularly and in many bulls is completed only after the onset of erectile activity. Thereafter, incomplete separation is defined as a persistent penile frenulum (otherwise known as a persistent raphe or tied penis). This condition, most commonly present in Angus, Beef Shorthorn, Hereford, Polled Hereford, and Beefmaster bulls, probably has a genetic basis in many cases. Although this condition is usually correctable with minor surgery, the consequences of perpetuating a genetic defect should be considered when this is done.

The prepuce is a double invagination of skin, with its internal lining everting upon penile erection to constitute much of the penile surface. A fan-shaped protractor prepuce muscle raises and lowers the distal portion of the prepuce and also controls the size of the preputial opening. Retraction of the membrane lining the inner prepuce is under the control of the retractor prepuce muscle. Lack of development of this muscle (a condition genetically linked with the polled gene in bulls), predisposes to chronic eversion of this membrane with increased risk of traumatic injury. The development and normal function of the accessory glands depend upon the effects of androgens. Castration results in marked depression of both development and secretory functions of these glands.

As spermatozoa progress through the epididymis, they achieve progressive motility, the cytoplasmic droplet migrates from the proximal to the distal position on the sperm midpiece, and seminal fluids are resorbed and exchanged. Heat stress can adversely affect epididymal function. The epididymis, particularly the tail region, acts as a storage region for sperm, such that a mature Holstein bull may have epididymal reserves representing the equivalent of 6 or 7 days of daily sperm output. Most sperm that are not ejaculated are voided in urine, with a small proportion resorbed by the male organs. Some evidence exists for selective resorption of spermatozoa within the epididymis. Sperms are transported from the cauda epididymis to the urethra in the ductus deferens (vas deferens) via muscle contractions that are strongest during precoital stimulation. The terminal portions of the ductus deferens expand to form the ampullae. These ampullae act as minor sperm storage areas and they also secrete fructose and citric acid into the seminal plasma. The ductus deferens open (via the ampullae) into the cranial portion of the pelvic urethra. The vesicular glands (or seminal vesicles) also open into the pelvic urethra. These glands provide much of the fluid component of the bull ejaculate, as well as sperm nutrients and semen buffers. The vesicular glands are lobulated organs, approximately 10 to 15 cm in length and 2 to 4cm in diameter in mature bulls. The prostate gland, consisting of a relatively small body and larger disseminate region, produces 25% to 40% of seminal volume as well as semen odor. The bulbourethral glands of the bull each open into the pelvic urethra at the ischial arch. The

urethra is an elongated tube extending from the bladder to the tip of the penis. It is surrounded by the urethralis muscle, which contracts strongly during ejaculation.

The function of male reproductive organs is under the control of both the nervous and endocrine systems. The latter includes the hypothalamus, anterior pituitary, and the Leydig and Sertoli cells. The hypothalamus plays a pivotal role in the control of reproduction, acting as the interface between the nervous and endocrine systems. It works within a complicated feedback system (the hypothalamic-pituitary-gonadal complex or axis), which is ultimately controlled by the inputs from the higher nervous system. In turn, this can be influenced by many factors, both environmental and behavioral. Gonadotropin-releasing hormone (GnRH) from the hypothalamus travels (via the portal system) to the anterior pituitary gland where it stimulates the synthesis and release of both LH (luteinizing hormone) and FSH (follicle-stimulating hormone). Leydig cells which located within the interstitial tissue (in close apposition to lymph and blood vessels), produce episodic bursts of T (testosterone) in response to LH release. Recently, the role of growth factors has been investigated in relation to male reproductive processes. Such factors act by stimulating target cell proliferation and regulate growth of reproductive organs. They include cytokines, interferons, insulin, IGFs, and others such as platelet-activating-factor (PAF) and epidermal growth factor (EGF). In the male, such factors have been associated with epithelial and interstitial cell development and function, puberty, LH modulation, and sperm motility. This represents a dynamic research area, with new findings appearing regularly to improve our understanding of the fine tuning of male reproduction.

11.3.2. Puberty and Sexual maturity

Puberty in bulls is a process that implies the attainment of functional sexual organs and behavior. Sexual maturity occurs when the development of both spermatogenesis and reproductive behavior allow effective coordinated service and subsequent fertilization. In prepubertal bulls, there is an early rise in gonadotropins (mainly LH) between 10 and 20 weeks of age. The earlier this rise in gonadotropins occurs, the earlier the onset of puberty. Spermatogonia start to appear in tubules within approximately 8 to 14 weeks after birth, with spermatocytes appearing shortly afterward. Seminiferous tubules form lumens between 15 and 40 weeks. Sequential maturation of spermatogonia through primary and secondary spermatocytes to spermatids and spermatozoa is achieved between weeks 32 to 44 in well-fed *Bos Taurus* breeds. Testicular growth is very rapid between 7 and 10 months of age. Blood concentrations of androgens start to increase at about 6 months of age and continue to rise through puberty (until at least 13 months of age). Puberty is often defined as the first time a bull produces an ejaculate with at least 50 $\dot{x}10^6$ spermatozoa per milliliter with at least 10% progressive motility.

11.3.3. Sexual behavior

The bull is attracted by the sight of mounting activity, particularly if the mounted female displays immobility. Here, visual cues are usually of greatest importance to the bull, with olfactory cues playing a secondary role. The bull may test the female for immobility by chin resting and sham mounting attempts. When more than one female is receptive, the bull will often preferentially mount the one that most recently came into estrus. Bulls may service an estrous female repeatedly, depending

upon his libido, stimulus pressure, and the length of time that the female remains receptive. Bull libido is subject to various influences including physical problems such as lameness, obesity; presence of a hernia, penile abnormalities, and illness. High-energy diets may reduce bull libido, whereas underfeeding is probably deleterious only when severe enough to affect the physiologic well-being of the bull.

11.3.4. Breeding Soundness Evaluation

It is estimated that at least one in five bulls in an unselected population would be subfertile owing to inability to serve cows efficiently or to poor semen quality. A bull requires three attributes to be fertile: (1) good libido, (2) physical soundness, and (3) good semen quality. These three attributes must be held in the forefront of all decisions regarding herd sire selection and breeding soundness evaluations. Bulls to be tested are exposed to restrained cows in a small paddock or pen and observed for expression of sex drive, ability to serve cows, and the number of services completed in a given time period.

A great deal of emphasis must be placed on sound conformation of the feet and legs of a bull. Bulls must be adequately restrained to allow comfortable and thorough palpation of the scrotum and its contents. A visual appraisal of the shape of the scrotum in a warm environment, while the bull is relaxed, reveals valuable information about the thermoregulatory abilities of the scrotum, as well as giving an indication of testis size. Presence of a scrotal "neck" above the testes is of critical importance because this region contains the countercurrent heat exchange mechanism of the testicular cords. In cooler temperatures, the scrotal shape cannot be determined, because the dartos muscle in the scrotal wall and the cremaster muscles will hold the testes closer to the body wall. In cool conditions, the testes must be manually pushed down into the scrotum, stretching the puckered scrotal wall to allow an assessment of scrotal shape. The scrotum also should be examined for thickness of the scrotal wall, the amount of fat in the neck of the scrotum, and lesions in or on the scrotum. The testicular cords should be palpated from the body wall down to the top of the testes to detect abscesses, varicoceles, or a scrotal hernia. The caput epididymidis, located primarily craniodorsally on the testis, usually is palpable and may feel more prominent in some bulls than in others. It is not uncommon to find enlargements in this area due to inflammation or sperm granulomas, which may prevent sperm transport and result in a small, flaccid, empty cauda epididymidis. The body of the epididymis can be palpated on the medial aspect of the testis by first sliding the opposite testis upward; however, it is extremely rare to detect abnormalities in the corpus epididymidis. The cauda epididymidis of a normally functioning testis is turgid and prominent. Differences in size and consistency between the left and the right cauda epididymidis may indicate inflammation on one side or may result from a blockage of sperm transport on the side of the smaller cauda. Segmental aplasia of one or both epididymides probably is an inherited condition. The testes must move freely within the scrotum. Careful palpation of the testes must be done to detect possible abscesses, tumors, hematoceles, or calcification. In some cases, ultrasonography may be helpful for diagnosis of testicular abnormalities. The consistency of the testis often is difficult to ascertain by subjective palpation. Although the use of tonometers for measuring consistency of testes removes much of the subjectivity, tonometer measurements have not been strongly correlated with semen quality and are not commonly used. In

general, yearling bulls have very firm testes compared with those of older bulls. Testes that are obviously soft are most likely to indicate testicular degeneration. This usually can be confirmed by semen analysis.

Scrotal circumference measurements are highly correlated with paired testis weight, which in turn is directly and highly correlated with daily sperm production and high semen quality traits.

Transrectal internal examination is used to palpate the accessory sex glands and the inguinal rings. The accessory sex glands of the bull include the prostate, bulbourethral, and vesicular glands. The vesicular glands are lobular structures 8 to 15 cm long, 3 to 5 cm wide, and 1 to 2 cm thick. They lie lateral to the ampullae and neck of the bladder. Congenital defects of the vesicular glands have been reported. These defects usually are unilateral and include aplasia, hypoplasia, cysts, and duplication of the gland. The bulbourethral glands are embedded in the urethralis muscle caudally near the anal region and are not palpable. The urethra usually is the first structure palpated. It is a firm tubular structure that usually becomes pulsatile on transrectal palpation as a result of the contractions of the urethral muscle surrounding it. The prostate gland is palpated as a transverse, smooth band surrounding the cranial extremity of the urethra. At this point, the vesicular glands can be palpated craniolateral to the prostate. The vesicular glands vary a great deal in size between bulls. The ampullae are not very distinct on palpation but can be found by pressing the fingertips over the floor of the pelvis cranial to the prostate gland and moving the fingers back and forth in a lateral direction. Enlarged inguinal rings are uncommon and are, in most instances, unilateral. A bull with an enlarged inguinal ring will be predisposed to development of a scrotal hernia during breeding.

The four categories of breeding soundness are satisfactory, questionable, decision deferred, and unsatisfactory. Bulls classified as satisfactory potential breeders are those that have met the minimum requirements for physical soundness and semen quality. Bulls that have unknown sex drive and mating ability may be classified as satisfactory, but documentation of the animal's breeding soundness must mention that this aspect needs to be examined by the producer. The questionable category is for bulls likely to perform adequately in mating but with below-normal fertility, or with an undesirable trait with the potential for genetic transmission to offspring.

11.3.6. Reproductive disorders

Lack of libido is difficult to measure or diagnose. Lack of libido may be due to inheritance, inexperience, or back, feet, leg, or joint abnormalities. Lack of libido is not treatable unless a primary medical cause is identified.

Orchitis is infrequently diagnosed in the bull. Orchitis generally is considered to be due to infectious causes. Infectious agents may reach the testicles by hematogenous spread from other loci of infection within the body. Infections in other parts of the reproductive or urinary system may extend to involve the testicles. Orchitis may result from wounds that penetrate the scrotal skin. Bacteria, including *B. abortus, Arcanobacter,* and others, are most frequently isolated in cases of orchitis. Certain viruses also may initiate testicular inflammation. The swelling associated with inflammation coupled with an inelastic tunica albuginea leads to pressure necrosis of the testicles. Thrombosis of

blood vessels and heat produces degeneration. The testicle rapidly loses its ability to function normally. The diagnosis of orchitis in the bull is straightforward. Observation of the scrotum shows one testicle larger than the other. On palpation, the scrotal contents will be painful, hot, and edematous. Ultrasound examination and thermography are useful aids in diagnosis. Medical treatments of orchitis may not be completely successful in returning the testicle to normal function. The contralateral testicle is at risk for degenerative changes if resolution of the inflammation is delayed. Antibiotics are of limited value in most cases. Cold water hydrotherapy may be of help. Often, bulls of moderate value are culled. If the bull is more valuable and the rest of his reproductive system is normal, surgical removal of the affected testicle will allow the other testicle to eventually compensate.

Testicular hypoplasia is defined as testis size smaller than normal for age; one or both testicles may be affected. Testicular hypoplasia generally is considered to have a large heritable component. Bulls with small testicles have reduced sperm motility, a lower percent of sperms with normal morphology, and less concentrated semen with fewer total sperm numbers per ejaculate. Affected animals may be subfertile or infertile. Libido is unaffected. This problem cannot be corrected, and affected animals should be culled.

Failure of normal testicular descent is referred to as cryptorchidism. This problem is relatively uncommon in bulls and usually is unilateral. Because of the possibility of inheritence, cryptorchid animals should not be used for breeding.

Testicular degeneration is an acquired condition in which testicles that were once normal undergo pathologic changes, resulting eventually in small testicular size and abnormal function. Testicular degeneration may affect one or both testicles, and the changes may be temporary or permanent. Increased temperature of the scrotal contents, even for a relatively short period of time, has been shown to result in testicular degeneration with a higher percentage of abnormal ejaculated sperm or even azoospermia. Fever, high environmental temperature, inflammation of the scrotal skin, and excessive scrotal fat all have been shown to have an adverse effect on testicular function, presumably secondary to increased testicular temperature. Extreme cold resulting in scrotal frostbite is reported to lead to testicular degeneration. Orchitis, testicular trauma, can result in degeneration of the testicles. Degeneration can result from blockage of parts of the excurrent duct system, such as the epididymis. Bulls seem to undergo testicular degeneration associated with advancing age earlier in life than other animals. Most bulls will undergo changes by 8 to 10 years of age. Diagnosis of testicular degeneration most often is based on the history, a careful examination of the scrotal contents including scrotal circumference measurements, and semen analysis. Ultrasound examination also has been used. Treatment is limited to removal of the cause of the degeneration when it can be determined. Re-examination at 45- to 60-day intervals will determine if the changes are temporary or permanent.

Epididymal inflammation, or epididymitis, in the bull most commonly is unilateral and involves the tail of the epididymis. Epididymitis may be diagnosed alone but often is seen associated with vesiculitis or orchitis. Infection with bacteria including *A. pyogenes* and *B. abortus* is the most common cause of epididymitis in the bull. Epididymitis often results in infertility secondary to obstruction of the lumen. Thermal injury to the testicle also may result. Inflamed epididymal tails are

hot, swollen, and painful early in the course of the disease but chronically become small, hard, and misshapen. Treatment generally is of little value, and most affected animals are culled.

Congenital absence of part or all of the epididymis usually is unilateral in the bull. This segmental aplasia may be hereditary, and affected animals should not be used for breeding. Segmental aplasia may be accompanied by enlargement of the duct system proximal to the missing area due to sperm stasis. Often, the corresponding vesicular gland or ampulla will be found to be absent.

Inflammation of the vesicular glands (seminovesiculitis) most frequently is diagnosed in bulls younger than 2 years of age or older than 9 years. The incidence of vesiculitis usually is low A number of bacteria and viruses have been associated with this problem. *Brucella abortus, Arcanobacter pyogenes, Haemophilus somnus,* and others have been described as bacterial causes of vesiculitis. The IBR virus and enteroviruses have been incriminated. The pathogenesis of vesiculitis has not been clearly established. A number of risk factors for this condition have been reported. Occasionally, signs of abdominal pain or rear leg lameness may be noted. More often, this disease is diagnosed during a routine breeding soundness evaluation or during examination of the bull as a part of a herd infertility investigation. Considerable variation in vesicular gland size, consistency, and texture is normal in bulls. Consequently, the interpretation of findings on rectal examination may be difficult. Inflamed vesicular glands are enlarged, painful, and firm. Loss of lobulations may be noted. The vesicular glands may be adherent to adjacent structures. Abscessation can occur. Changes may be unilateral or bilateral. The ejaculate of affected animals may be darker than normal in color and contain clumps. Medical treatment of this condition is based on long-term antibiotic therapy.

Fibropapillomas are the most common mass seen on the bull's penis and have a viral etiology. The causative virus is believed to gain entry into the preputial epithelium through abrasions of the penis associated with mating activity among bulls. This problem usually is seen only in young bulls and is not associated with warts on other parts of the body. Phimosis or paraphimosis may result from the presence of larger masses. Penile fibropapillomas may occur as single or multiple masses. The mass usually is pedunculated, but sessile lesions with a diffuse distribution also are possible. Fibropapillomas sometimes will regress without treatment. Surgical removal of pedunculated masses is straightforward. Commercial wart vaccine has been used in treatment, with variable results. Commercial autogenous wart vaccine may be more useful in preventing recurrence of the problem, which may occur in up to one third of affected animals.

Persistent penile frenulum is a congenital band of tissue extending from the median raphe of the prepuce to the ventral side of the penis near the glans. Penile erection and extension in affected animals results in a ventral bowing of the penis. In bulls with longer prepuces, the prepuce may be pulled over the glans penis by the band. In either case, successful intromission is unlikely. This condition is believed to be heritable, and affected animals should be used only as terminal sires. Surgical correction of persistent penile frenulum requires that the penis be extended and secured. Local anesthetic injection is given at each end of the band. Blood vessels and the band are ligated, and the band is transected and removed. Healing occurs rapidly.

Inflammation of the penis and prepuce occurs occasionally in younger bulls. Pain resulting from this inflammation may be severe enough that the affected bull mates less often or not at all. Most frequently, this condition is associated with abrasions occurring at mating or infection with bovine herpesvirus type 1 (BHV-1). Inflammation of the penis and prepuce may be severe enough to produce adhesions, with limitation of future fertility. In most cases, however, the problem is self-limited and will resolve after 2 weeks of sexual rest.

Hair rings encircling the penis may be seen, especially in younger bulls that are housed in groups. It is believed that these hair rings result from mating activity during which the penis is rubbed across loose body hair. Eventually, a ring of hair may accumulate on the penis, resulting in discomfort, necrosis, and occasionally, urethral fistula or penile amputation. Removal of the hair ring and local wound therapy should result in the resolution of reversible pathologic changes.

Penile hematoma occurs as a result of rupture of the tunica albuginea associated with injury at breeding. Affected bulls most often are Polled Hereford and show reluctance to mate, a prolapsed prepuce, and a swelling anterior to the scrotum, which cannot be separated from the penis. Paracentesis should not be used for diagnosis because 60% of the cases progress to abscess formation. Approximately 70% of bulls with hematoma smaller than 20cm in diameter will recover if given antibiotics and 6 months of sexual rest. About 70% of bulls with hematoma greater than 20cm in diameter will recover with suturing of the torn tunica albuginea and 3 months of sexual rest. About 50% of bulls with hematomas larger than 20cm will recover with antibiotics and sexual rest.

11.4. Buffalo-Bulls

11.4.1. Anatomy and physiology of the reproductive organs

The reproductive organs of the buffalo bull are quite similar to that of domestic cattle bull. However, the scrotum is conical in shape, wrinkled and even pigmented. Testicular weight, a reliable index of semen producing ability, has been shown to increase between 2.5 - 3.0 (68.5 g), 3.5 - 4.0 (96.2 g) and 4.5 - 5.0 (114.2 g) years of age. Concerning seasonal effects, neither testicular weight, parenchyma weight nor the number of sperms produced by testis were season –dependent. The epididymis of the buffalo is a rather intricately convoluted duct with complicated structure. It is much shorter than that of the bull. The caput and proximal corpus are the most active sites at which maturation of spermatozoa is likely to occur.

The gross appearance of the vesicular glands resembles that of the bull except for the pattern of lobulation and the shape of urethral end. These glands are markedly smaller than in Bos taurus bulls of comparable age as regards their size and weight. These glands show rapid growth during the first 1.5 years of age but growth rate slows down till the age of 3.5 years when it appears to cease completely. There is an indication, however, that the glandular epithelium is significantly higher at 3-4 years than at 2-3 years of age which could entail not only higher secretory activity but also age differences in the response of glandular epithelium to male sex hormone. The length of the ampullae of the ductuli deferentis testis does not vary significantly with age although their diameters do.

External prostate of the male buffalo is ill-developed and resembles that of *Bos taurus* bulls in histological features. The pars interna is well developed and can be easily peeled off the surrounding urethral muscle (length= 6.7 cm, proximal width= 3.9cm, distal width= 2.9cm, thickness = 2.4cm, weight= 46.7 g). In this portion of prostate gland, two types of secretory units (arbitrarily termed A and B) are present. These are different in their location, frequency, size height of epithelium, cytoplasmic affinity to staining and nature of secretory material present therein. Bulbo-urethral glands do not vary significantly in weight or dimensions in bulls aged 2-4 or more years.

11.4.2. Puberty and sexual maturity

The age of puberty and first ejaculation in Egyptian buffalo bulls occurs by about 14.2 months. Indian workers believe that initiation of sexual function is influenced by season, independent of age. However, in spite of early puberty, bulls in Egypt are put to service at about 3 - 3.5 years of age. Appropriate feeding and management of prepubertal buffalo bulls are thought to be of value in enhancing puberty, since first signs of sexual interest and meiotic divisions of spermatogonial cells were found to occur as early as 9 months. During the first postpubertal year, a marked increase in the sperm producing capacity has been reported.

11.4.3. Sexual behavior

Sexual behavior in buffalo-bulls is less prominent than in that of cattle-bulls. Data on 214 ejaculates of six adult Murrah buffalo bulls have been used to study the effects of seasons on sexual behavior. Significant seasonal variation was obtained in reaction time, total time taken for successful ejaculate, dismount time and libido score. Reaction time and total time (in seconds) for ejaculate were highest during summer (89.56±10.58 and 151.25±18.64) and lowest during winter (42.67±8.63 and 92.81±20.90). Libido score was lowest during the summer (5.06±0.22) and highest during the rainy (6.81±0.39) season; however, the difference between the winter and rainy seasons was not significant. It was concluded that the sexual behavior and semen qualities were optimal during winter, poor during summer and intermediate during the rainy season.

In Italy, male buffaloes are generally considered to be potentially active and fertile all the year round, though some seasonal differences in gonadotrophin release and sperm quality have been reported. Semen quality and freezability were also poor in very poor and poor libido bulls. Testosterone level was not associated with poor libido.

11.4.4. Breeding sound evaluation

Breeding soundness examination is an effective way to select potential fertile breeder bulls and to cull sterile bulls before their entry into the breeding unit. BSE includes records of breeding history and systemic illness of bull; general clinical evaluation; examination of circulatory, respiratory, digestive, urinary and genital systems. Sexual behavior and semen evaluation are also recorded during breeding soundness examination.

11.4.5. Reproductive disorders

In one study, twenty-two buffalo bulls suffering from three different types of infertility were slaughtered. Except for the reproductive system, no signs of localized or generalized disease were observed. Microbiological investigations were negative for brucellosis, vibriosis, mycoplasma and other non-specific microorganisms. Nine bulls with type I infertility had low bodyweights and underdevelopment of testes, accessory sex glands and endocrine glands. One bull of this type also showed bilateral epididymitis. Four out of 11 bulls with type 2 infertility had low bodyweights and most suffered from underdevelopment of testes, accessory sex glands and endocrine glands. Six bulls of this type had lesions of either epididymitis or orchitis or both. Two of these animals showed adhesions of periorchitis. One also showed seminal vesiculitis. In two bulls with type 3 infertility, bodyweights, reproductive organs and endocrine glands were normal. In later life, they yielded poor quality semen. Semen samples collected a few months before slaughter from nine bulls with type 2 and type 3 infertility were of poor quality and had higher percentages of abnormal spermatozoa in most cases.

In another study, forty-four buffalo bulls, used for artificial insemination, were studied to develop libido, mating ability and sexual behavior indices for selection purposes. For each index, 5 categories (excellent, very good, good, fair and poor) were established. The sexual behavior index was found to be more reliable than the libido and mating ability indices. Buffalo bulls in good to excellent categories were considered acceptable sires. Reaction time, sexual aggressiveness, and scores of libido, mating ability and sexual behavior differed significantly among the various categories of the 3 indices. Libido significantly correlated with mating ability. Sexual behavior expressed significant relationship with age ($r=0.41$; $P<0.01$) and body weight ($r=0.48$; $P<0.01$), but was nonsignificant with the scrotal circumference ($r=0.28$; $P>0.05$) of buffalo bulls. However, these relationships were absent ($P>0.05$) in the acceptable sires. Semen production was correlated with sexual behavior in only the fair and poor categories of buffalo bulls ($r=0.84$; $P<0.005$). Sexual behavior had no relationship with the fertility rate of buffalo bulls ($r=0.44$; $P>0.05$). It is concluded that the sexual behavior index can be used successfully for the selection of buffalo bulls. Excellent to good bulls should be used in an artificial breeding program if they are qualified in the other selection indices.

11.5. Rams and Bucks

11.5.1. Anatomy and Physiology of the reproductive organs

Likewise in bull, the male reproductive system consists of testicles, which produce sperm and sex hormones, a duct system for sperm transport, accessory sex glands, and the penis, or male organ of copulation, which deposits semen in the female. The testicles are relatively large, while the body of the prostate is absent.

11.5.2. Puberty and sexual maturity

Puberty is the age at which the ram's reproductive organs become functional, his secondary sex characteristics develop, and he is ready to successfully mate ewes. Most ram lambs reach puberty between 5 and 7 months of age, at 50 to 60 percent of their mature weight. The onset of puberty is affected by breed, genetics, and nutrition. Ram lambs on a low plane of nutrition may not reach puberty until they are 12 months of age or older. Some breeds reach puberty earlier than other breeds: prolific breeds and hair sheep. Meat breeds tend to reach puberty earlier than wool breeds.

11.5.3. Sexual behavior

The willingness to breed ewes is highly variable among rams and can have a major impact on sheep production, especially in a single-sire mating scheme. Libido is a ram's desire to mate. It is regulated by the release of testosterone, produced by specialized cells in the testes.

Some breeds of rams show libido almost continuously once they reach puberty. In other breeds, there is a marked decline in libido during the non-breeding season. Underfed and overfat rams may show reduced libido. A ram's desire to mate also decreases with age and disease conditions, such as arthritis.

11.5.4. Breeding Soundness Evaluation

Breeding soundness evaluation (BSE) is an overall assessment of the ram’s capacity for serving and impregnating a number of ewes during a breeding season. An overall physical examination is performed with special emphasis on the reproductive system. The BSE includes anatomic and structural correctness, freedom of disease, body condition, scrotal circumference, and semen quality. The ram accounts for the major genetic changes in a flock. A BSE includes an evaluation of ram management and an assessment of the potential genetic contribution of the ram.

Libido refers to the willingness of a ram to breed ewes. A serving capacity test or direct observation of a ram with ewes is an important component of the BSE, but is frequently neglected. It has been estimated that approximately 10% of all rams have no interest in breeding ewes.

The ram should be examined for conditions that may prevent optimal performance, or that can be transmitted to the ewes, such as foot rot, lip and leg ulcerations (ulcerative dermatitis), and pizzle rot. Body condition should also be noted during the physical examination. A body condition score of 2.5 to 3.5 is recommended for rams entering the breeding season.

The testes and epididymides should be examined for palpable gross abnormalities. Testicular tone and symmetry should be assessed. Swellings, atrophy, and lack of tone or symmetry often indicate pathologic problems and decreased fertility. The caput (head), corpus (body), and cauda (tail) of the epididymis are readily palpable in the normal ram. The most commonly encountered epididymal lesions usually involve enlargement of the epididymis due to inflammation and fibrosis. The major infectious agents resulting in epididymitis are *B. ovis* in mature, sexually exposed rams, and

Histophilus spp. Or *Actinobacillus* spp. in young, virgin rams. Other organisms, such as *Corynebacterium pseudotuberculosis*, can also produce epididymitis.

The prepuce should be free of any raw or ulcerative lesions at the orifice. Lesions can indicate conditions such as pizzle rot (sheath rot, ulcerative posthitis), lip and leg ulceration, or phimosis.

The penis should be extended and examined. If the penis cannot be extended, a previous injury or disease may have produced adhesions. The glans and urethral process should also be examined. Ulcerative dermatosis and urethral calculi can destroy the glans.

Semen analysis is an important step in Ram evaluation. Increased morphologic abnormalities of sperm cells are evidence of an insult on the reproductive organs. In many instances, it is difficult to determine whether such abnormalities are transient or permanent in nature. Spermatogenesis in the ram requires approximately 49 days. An additional 10 to 14 days are required for the spermatozoa to travel through the epididymis. During passage through the epididymis, maturation of the spermatozoa continues and several functional changes occur. These changes include the potential for sustained motility, the progressive loss of water, the distal margination and eventual loss of the cytoplasmic droplet, and development of the potential capacity to fertilize ova. Thus, semen collected on any given day was produced 59 to 63 days earlier. Therefore, the semen evaluation portion of the BSE should be conducted as near to the start of the breeding season as possible.

11.5.5. Reproductive disorders

Brucella ovis is an economically important cause of epididymitis, orchitis and impaired fertility in rams. Ovine epididymitis is caused by *Brucella ovis*, a Gram-negative coccobacillus or short rod. Rams often become persistently infected, and many of these animals shed *B ovis* intermittently in the semen for 2 to 4 years or longer. *B. ovis* can also be transmitted by direct non-venereal contact between rams. Ram-to-ram transmission is poorly understood and may occur by a variety of routes, including oral transmission. Epididymitis may be unilateral or, occasionally, bilateral. The testes may atrophy. Palpable lesions are often permanent, although they are transient in a few cases. Some rams shed *B. ovis* for long periods without clinically apparent lesions. *B. ovis* can also cause abortions and placentitis in ewes, but this appears to be uncommon. Infected ewes may give birth to weak lambs that die soon after birth. Systemic signs are rare in adult ewes and rams. *B. ovis* can cause poor semen quality in red deer stags, but abortions have not been reported in hinds. Lesions are mainly found in the epididymis, tunica vaginalis and testis in rams. The lesions vary from a slight enlargement of the epididymis to large indurations. Epididymal enlargement can be unilateral or bilateral, and the tail is affected more often than the head or body. Other bacteria that cause epididymitis and orchitis should be considered. Commonly isolated organisms include *Actinobacillus seminis*, *A. actinomycetemcomitans*, *Histophilus ovis*, *Haemophilus spp*., *Corynebacterium pseudotuberculosis ovis*, *Chlamydophila abortus* and *B. melitensis*, but many other organisms can also cause these conditions. Sterile, trauma-induced spermatic granulomas should also be ruled out.

Fertility may remain low even if the organism is eliminated. Infections in ewes are generally prevented by controlling infections in rams. *Brucella* species are readily killed by most commonly

available disinfectants including hypochlorite solutions, 70% ethanol, isopropanol, iodophores, phenolic disinfectants, formaldehyde, glutaraldehyde and xylene; however, organic matter and low temperatures decrease the efficacy of disinfectants. Disinfectants reported to destroy Brucella on contaminated surfaces include 2.5% sodium hypochlorite, 2-3% caustic soda, 20% freshly slaked lime suspension, or 2% formaldehyde solution (all tested for one hour). Boiling for 10 minutes is usually effective for liquids.

If the temperature in the testes cannot be kept low enough, due to hot weather, the production of viable sperm may be affected. Fully developed sperm are less affected by heat stress than sperm in the developing stages. The prevent heat stress; rams should not have a full fleece during the breeding season. They should be sheared 6 to 8 weeks prior to breeding. The scrotal sack should be free from wool. Adequate shade and water should be provided during the breeding season. In extreme circumstances, rams can be housed during the hottest part of the day and put out for breeding during the cooler parts of the day

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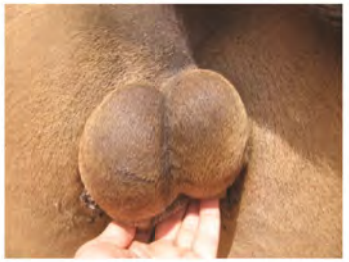
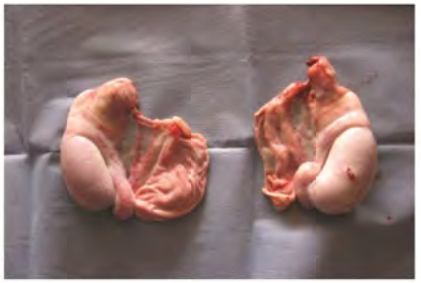
APPENDIX:Tables and Figures

Table 23: Comparative anatomy of the genital organs in male animals

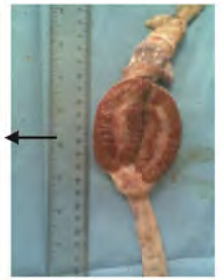
	Male camel	stallion	Bull	Buffalo-bull	Ram and Buck
Testes: position	Perineal – oblique	Prepubic – horizontal	Inguinal – vertical	Inguinal – vertical	Inguinal – vertical
Size	7-10x5x5 cm	8-11x3-6x3-6 cm	10-15x6-8x5-6 cm	8-12x4-5x4-5 cm	8-11x3-11x3-11cm
Weight	225 gm	300 gm	200-500 gm	150-300 gm	Ram:200-300gm Buck: 150 gm
Epididymis: position : parts	Anterior to testes Head, body, tail Body is the largest	Dorsal to testes No distinct parts	Caudomedial to testes Head, body, tail Tail is the largest	Caudomedial to testes Head, body, tail Tail is the largest	Caudomedial to testes Head, body, tail Tail is the largest
Vas deference relative to the testis	anterior	dorsal	caudomedial	caudomedial	caudomedial
Seminal gland	absent	12-15x5x5cm Pear-shaped, smooth	10-15x5x3cm S-shaped, lobulated	8-10,3.5x3.5 S-shaped, lobulated	3.5x2.5x2.5cm S-shaped, lobulated
Prostate gland:					
Pars externa	Discoid, 5x5cm	2 lobes, 5x3cm	3x1cm	3x1cm	Absent
Pars interna	Along pelvic urethra	absent	Along pelvic urethra	Along pelvic urethra	Along pelvic urethra
Bulbo-urethral gland	Ovoid, 2.5x1.5cm	Ovoid, 5x2.5cm	Circular, 2.5x1.5cm	Circular, 3x2 cm	Circular, 1.5x0.8cm
Penis: type	Firbro-elastic	Musculo-cavernous	Firbro-elastic	Firbro-elastic	Firbro-elastic
Glans penis	Hock-shaped	Circular	Spiral	Spiral	Crown, urethral process
Sigmoid flexure	Pre-scrotal	absent	Post-scrotal	Post-scrotal	Post-scrotal
Prepuce	Triangular, directed caudally	telescopic	Tubular, tuft of hair	Fleshy, no hair	Tubular, tuft of hair



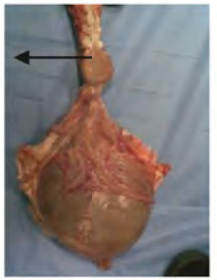
A) Slighter asymmetry between left (larger) and right testicles - normal.



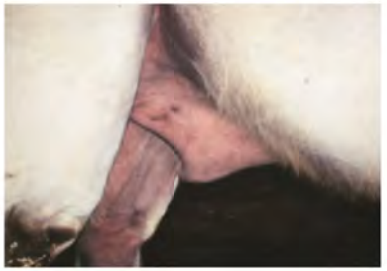
B) Post-mortem examination of camel testicles and epididymis.



C) Cross-section of camel testicles – pale central area (rete testis - arrow) surrounded by normal testicular tissue.



D) Body of the prostate (arrow) at the neck of the urinary bladder.

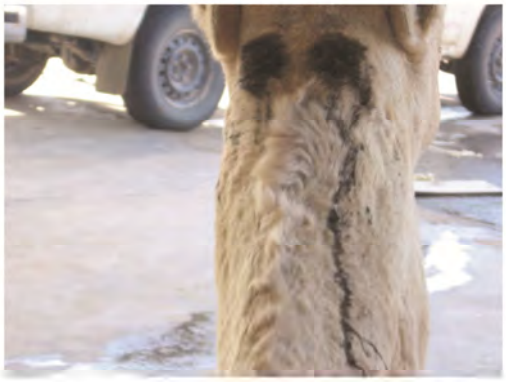


E) Prepuce – triangular shape directed backward

F) Adhesion of glans penis to the prepuce in an immature camel

G) Penis of a mature camel

Fig. 77: Anatomy of the genital organs of male camels (*Qassim-KSA, 2007-2013*).



A) Heavy secretion of poll gland (dark secretion) during breeding season

B) Taking the head backward

C) Keeping the two hind limbs away from each other with frequent urination

Fig. 78: Sexual behavior in male camels (*Qassim-KSA, 2010*).

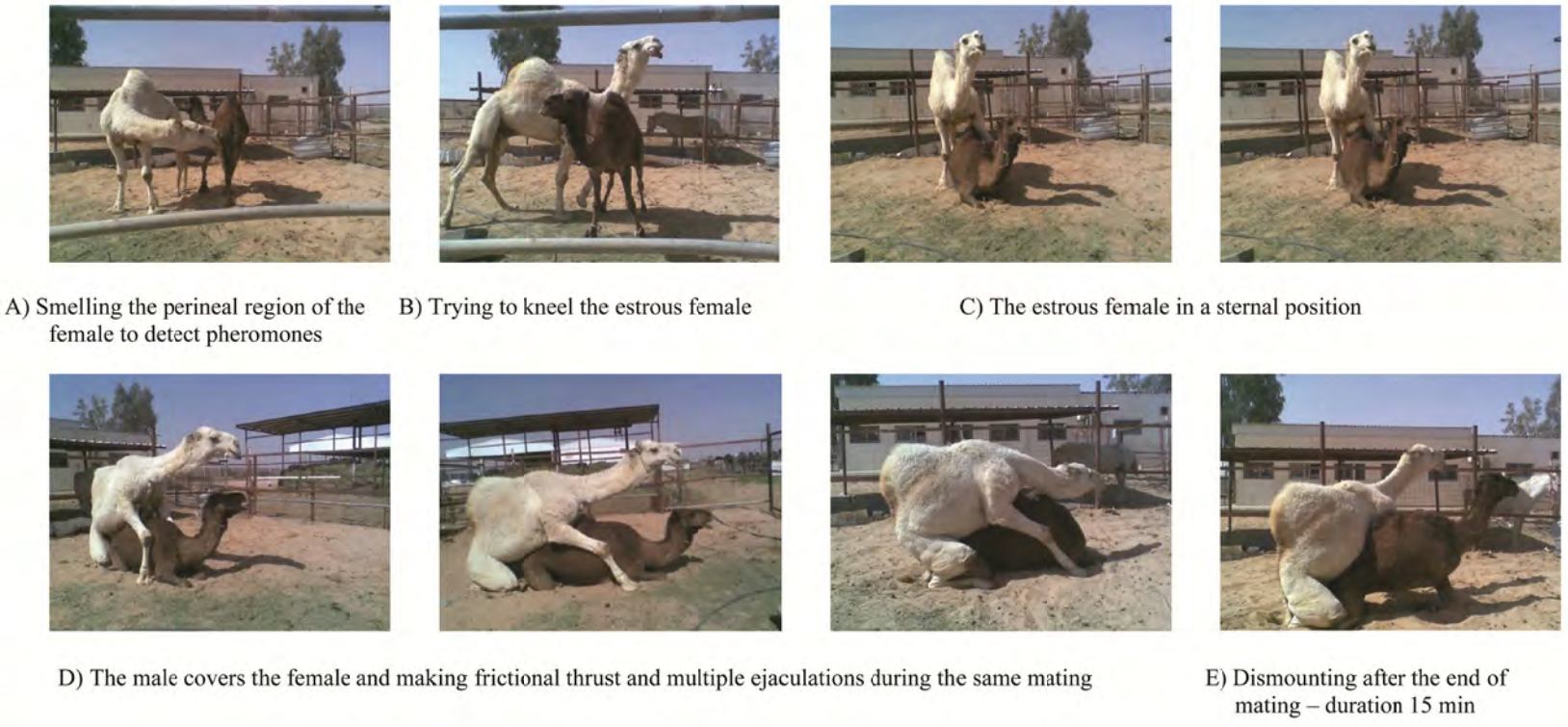
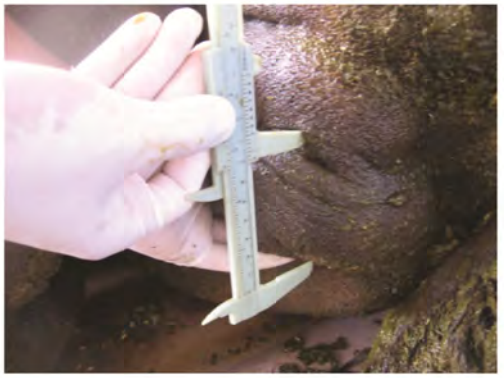
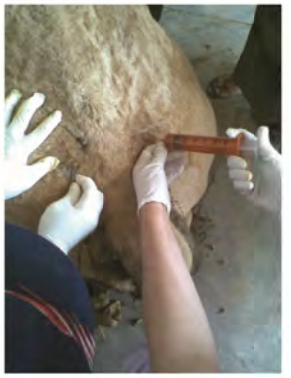


Fig. 79: Mating technique in male camels (*Qassim-KSA, 2012*).



A) Estimation of testicular dimensions using caliper.

B) Examination of the prepuce and penis.

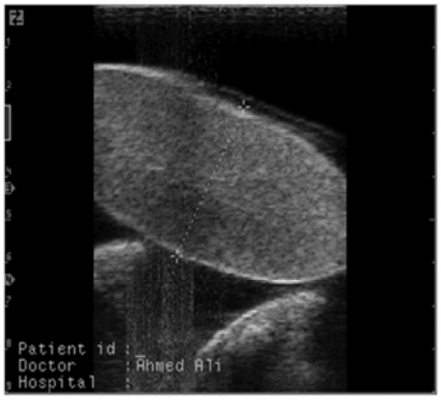


C) Pudendal nerve block for examination of the penis outside the prepuce.

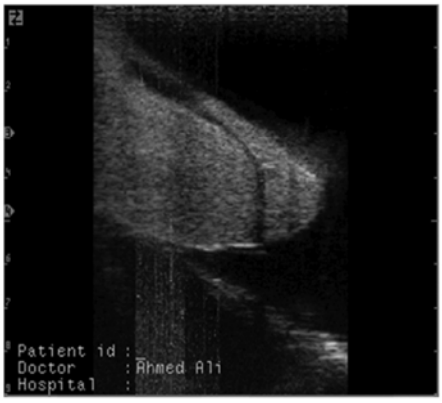
D) Exploration of the penis after application of the pudendal nerve block.

F) Biopsy needle for taking a testicular tissue samples

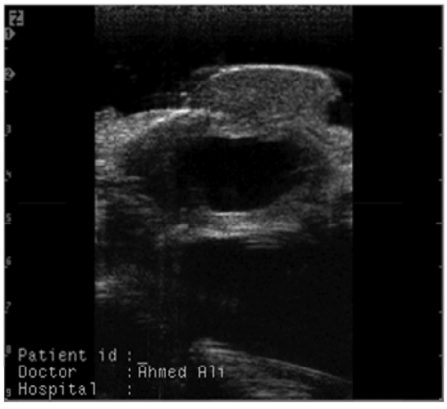
Fig. 80: Breeding soundness evaluation in male camels (*Qassim-KSA, 2007-2013*).



A) The testicles in longitudinal section



B) Testicle and epididymis



C) The prostate gland covers the urinary bladder.

Fig.81: Ultrasonography of the male genital organs in camels (*Assiut-Egypt, 2006*).

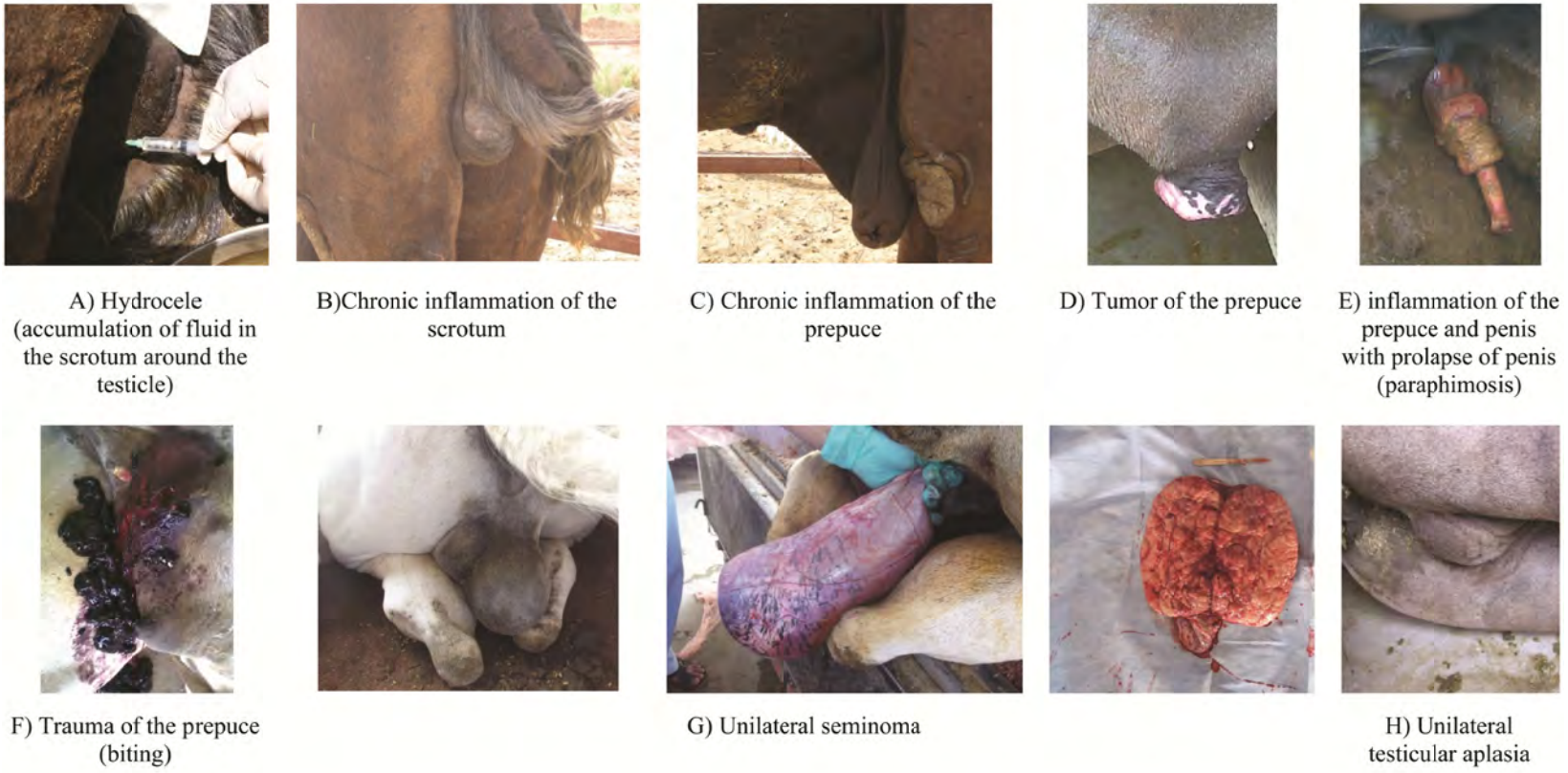
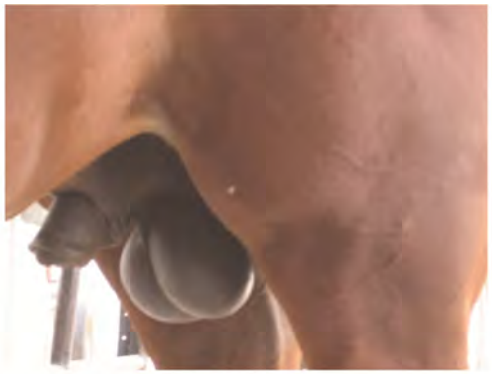
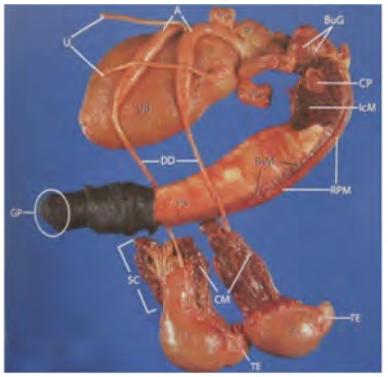


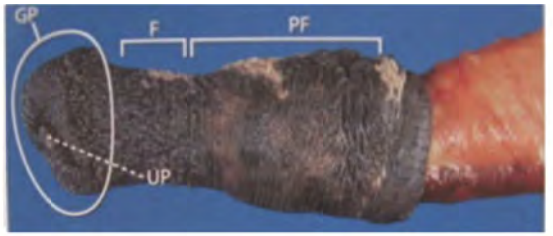
Fig. 82: Diseases of the genital organs in male camels (*Qassim-KSA, 2007-2013*).



A) External genitalia: two testicles, prepuce and penis

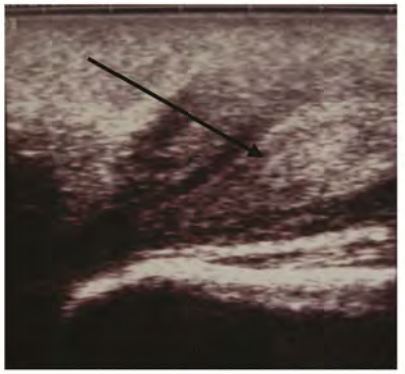


B) Internal (prostate, seminal, cowper's glands) and external genitalia

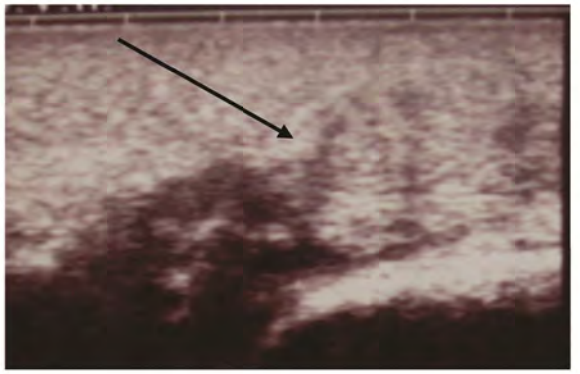


C) Free portion of the penis showing the shaft of the penis, the round glans penis, and urethral process.

D) Testicle: echogenic and homogenous



E) Prostate gland: two lobes



F) Cowper's gland: ovoid

Fig. 83: Morphology and ultrasonography of the genital organs in stallion (*Berlin-Germany, 1998; Senger, 2003; Qassim-KSA, 2009*).

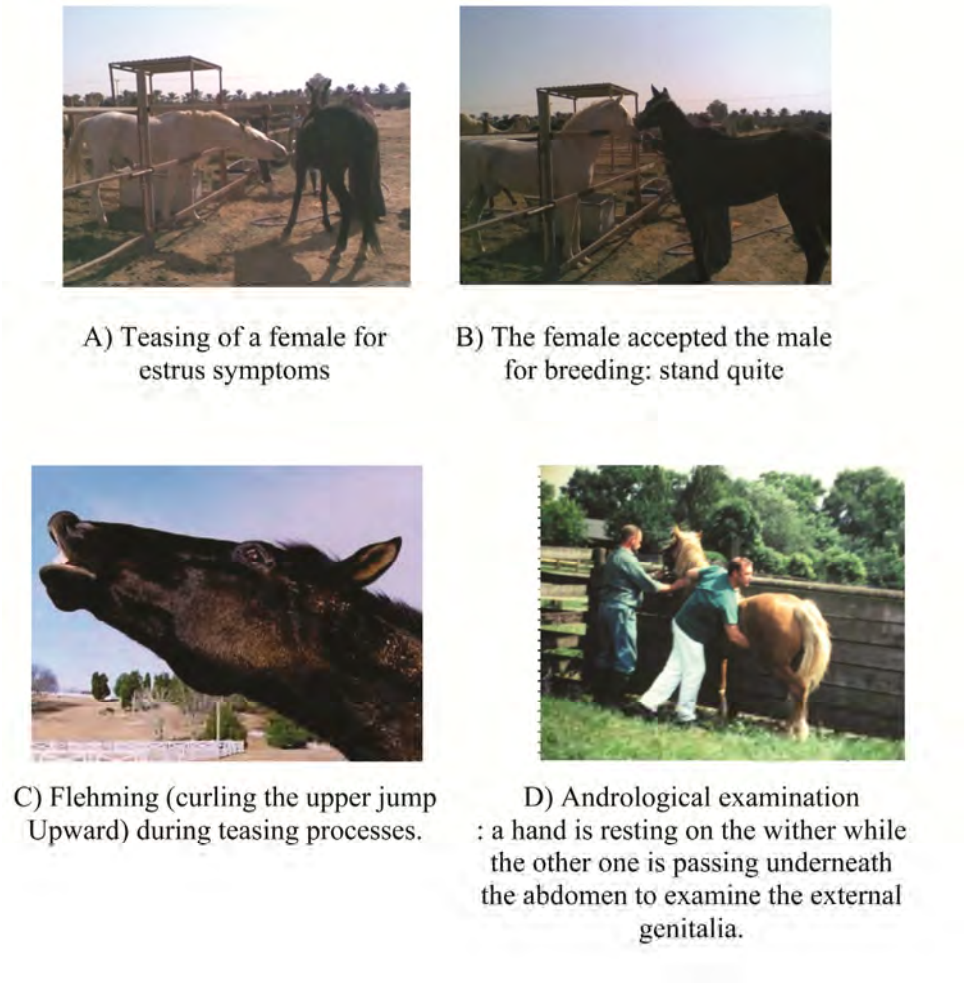
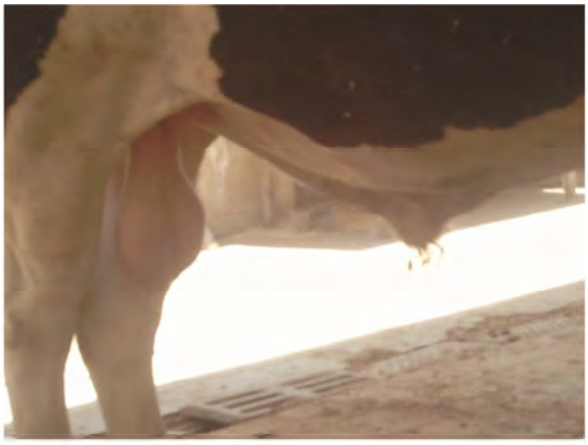
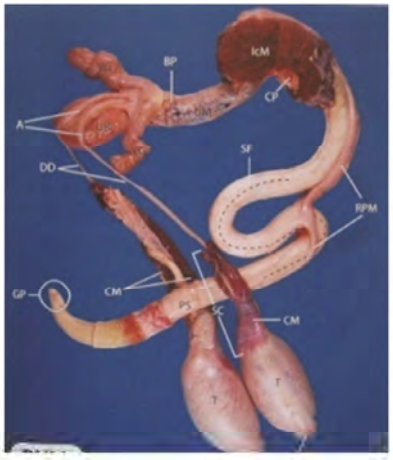


Fig. 84: Sexual behavior and breeding soundness examination in stallions (*Berlin-Germany, 1997; Qassim-KSA, 2010*).

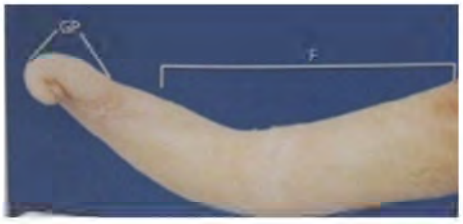
Fig. 85: Diseases of the genital organs in stallions (*Senger, 2003; Qassim-KSA, 2007-2013*).



A) The external genitalia (two testicles and Prepuce).

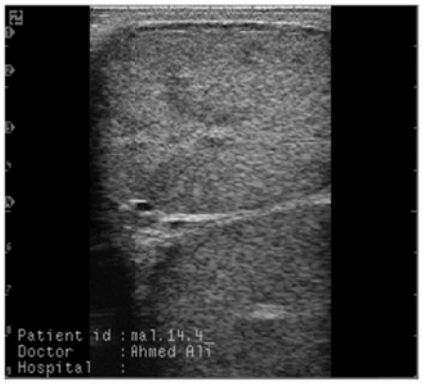


B) The internal (two seminal glands, body of the prostate, bulbocavernous muscle) and external genitalia (testicles, penis, retractor Penis muscles)

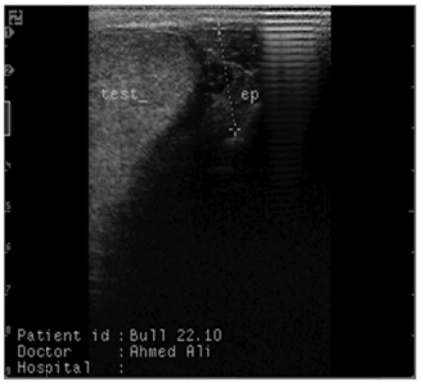


C) The free portion of the penis: the shaft and galea glandis.

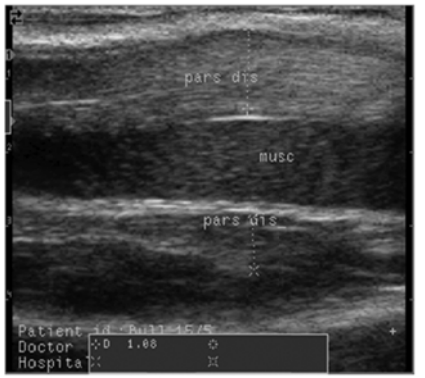
Fig. 86: Anatomy of the genital organs in bulls (*Senger, 2003; Assiut-Egypt, 2005*).



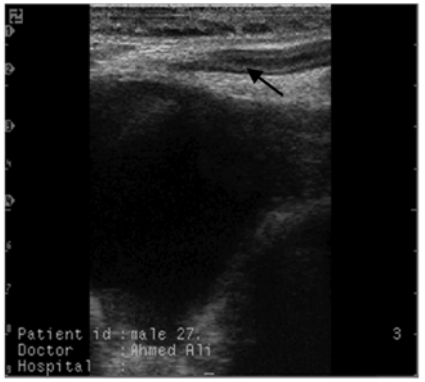
A) Testicles in cross-section: echogenic and homogenous with central echogenic area of mediastinum



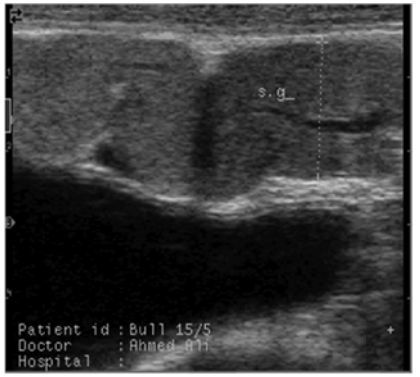
B) Tail of the epididymis



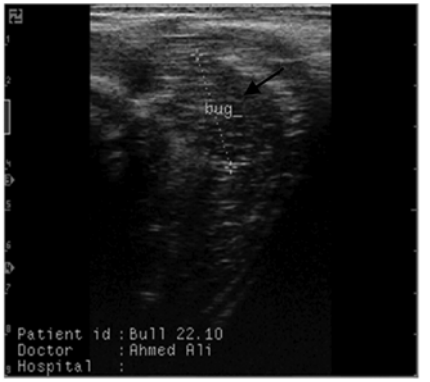
C) Pelvic urethra showing internal part of the prostate gland



D) Ampulla vas deference



E) Seminal gland



F) Bulbo-urethral gland

Fig. 87: Sonograms of the genital organs in bulls (*Assiut-Egypt, 2002*).

A) Holding the female with the two forelimbs and resting the body on the hindlimbs to make an ejaculatory thrust

Fig. 88: Sexual behavior and mating technique in bulls (*www.vetmed.Isu.edu*).

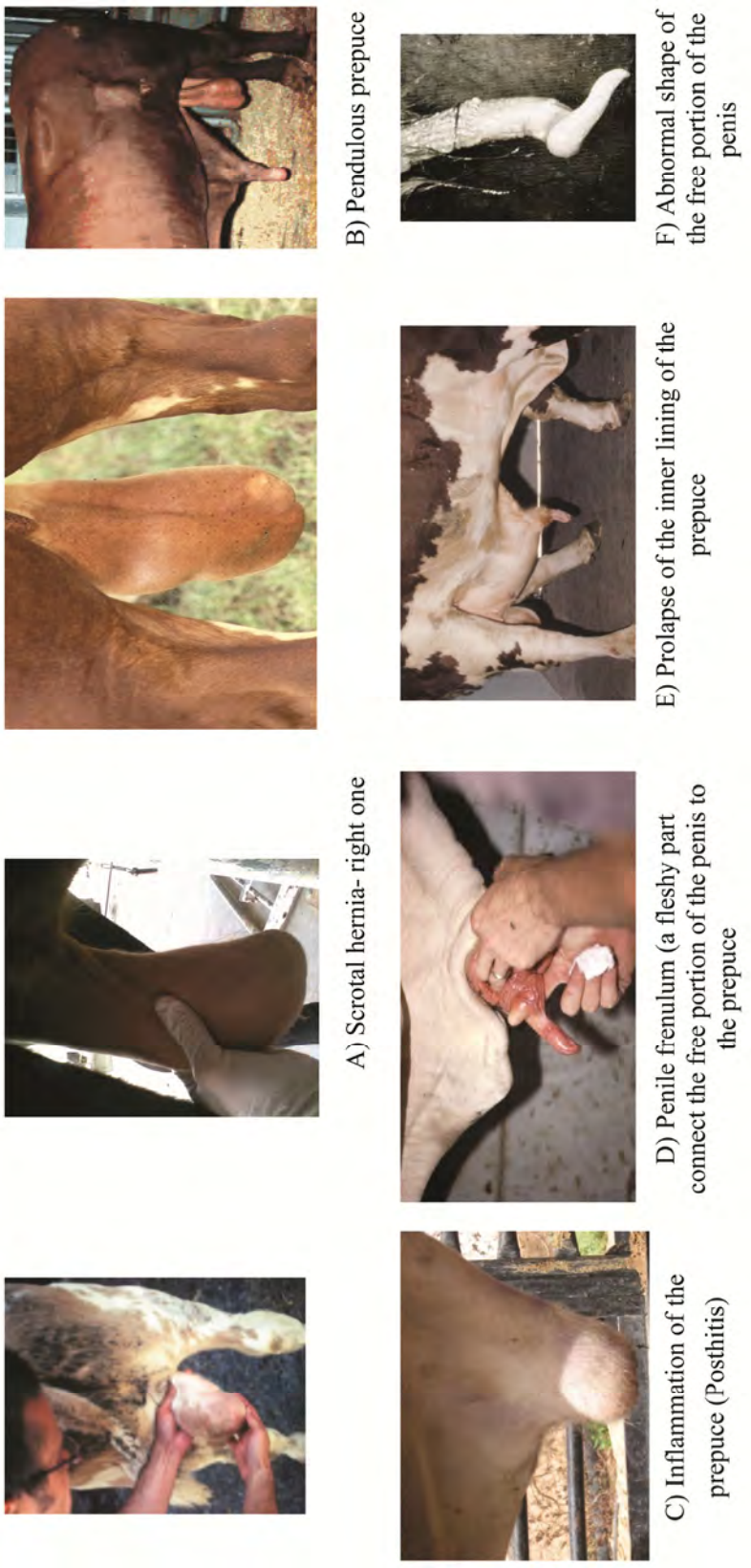


Fig. 89: Diseases of the genital organs in bulls (*Berlin-Germany, 1995-2000*).

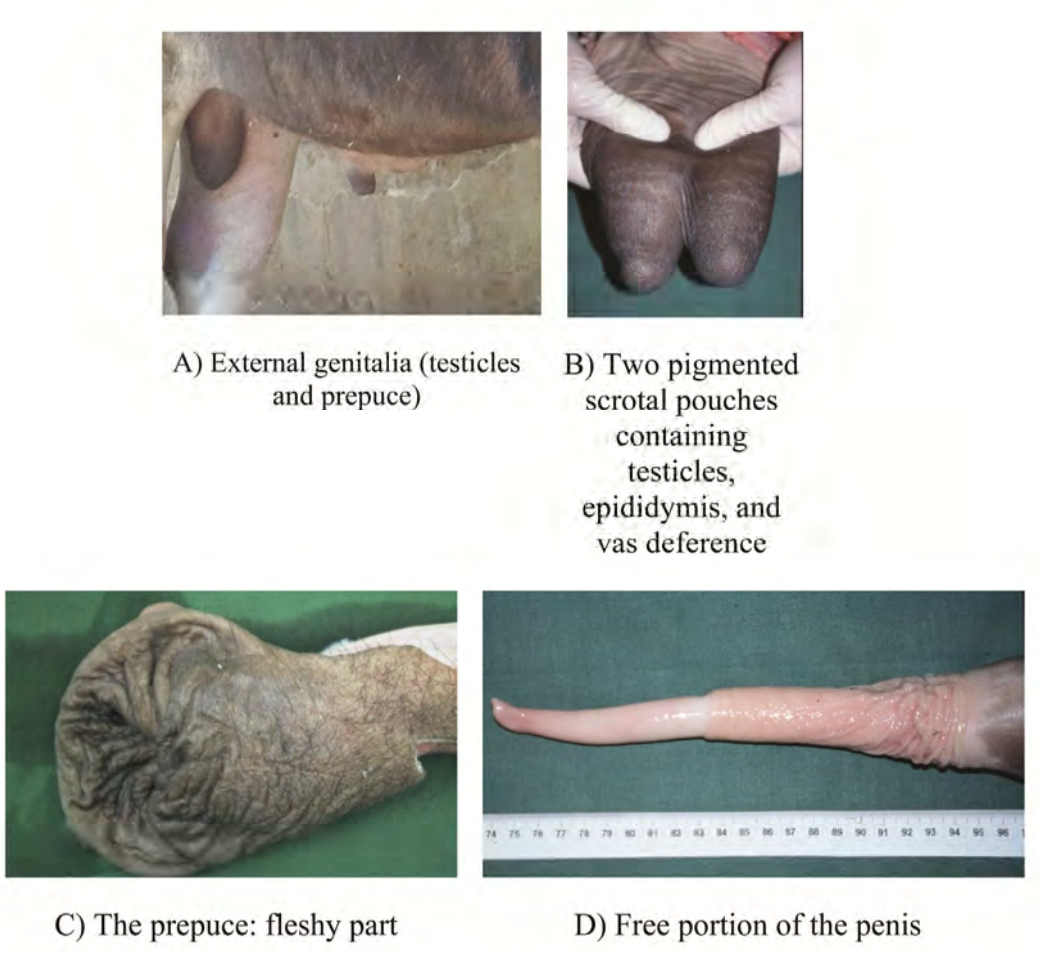


Fig. 90: Anatomy of the male genital organs in buffalo-bulls (*Assiut-Egypt, 2004*).

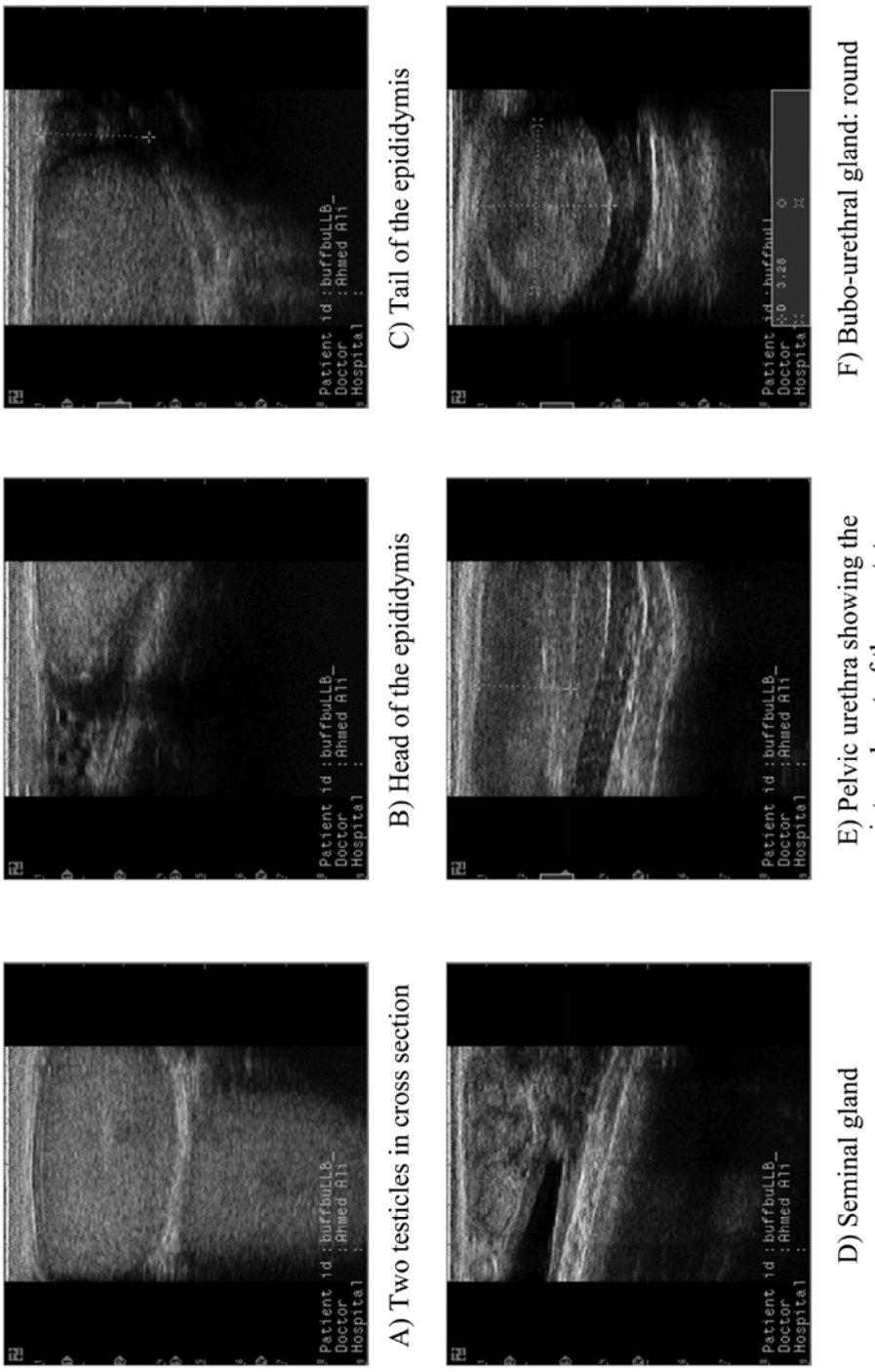
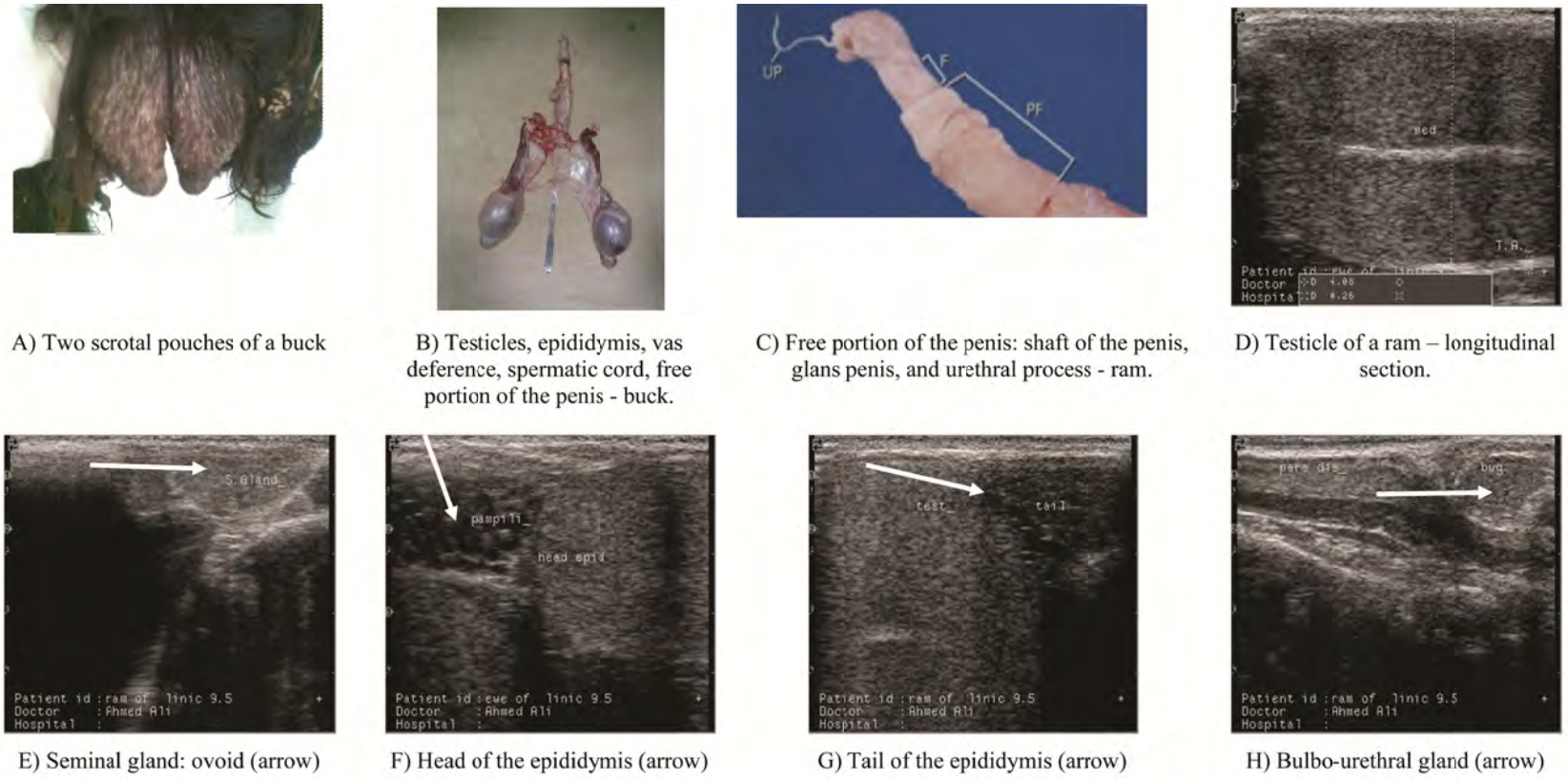


Fig. 91: Ultrasonography of the genital organs in buffalo-bulls (*Assiut-Egypt, 2002*).

A) Smelling of the perineal region, resting of the head on the female back, pushing the ground with the fore-limbs, holding the female with fore-limbs, and resting on the hind-limbs to make an ejaculatory thrust

Fig. 92: Sexual behavior in a buffalo-bull (*Assiut-Egypt, 2013*).



A) Two scrotal pouches of a buck

B) Testicles, epididymis, vas deference, spermatic cord, free portion of the penis - buck.

C) Free portion of the penis: shaft of the penis, glans penis, and urethral process - ram.

D) Testicle of a ram – longitudinal section.

E) Seminal gland: ovoid (arrow)

F) Head of the epididymis (arrow)

G) Tail of the epididymis (arrow)

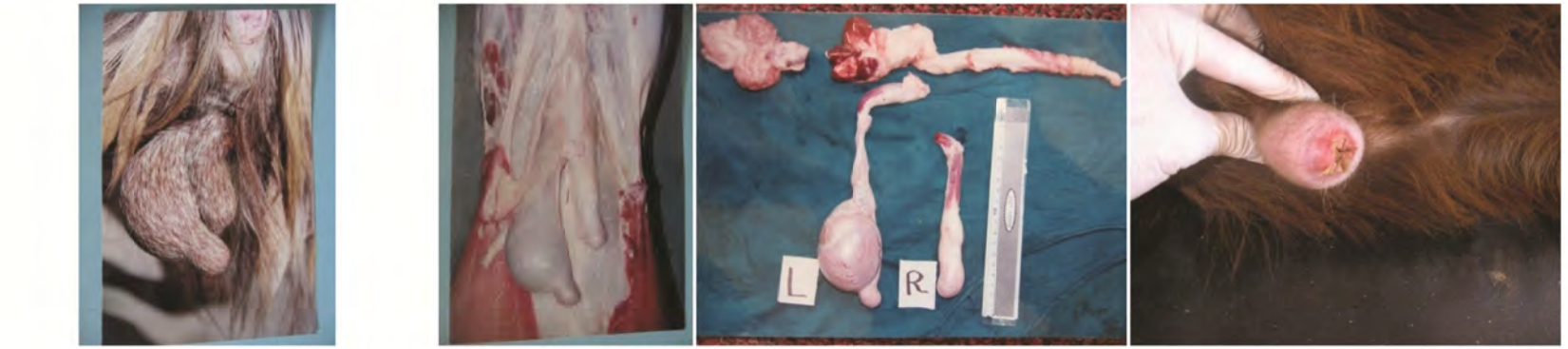
H) Bulbo-urethral gland (arrow)

Fig. 93: Morphology and sonography of the genital organs in ram and buck (*Assiut-Egypt, 2001; Senger, 2003*).

A) A ram searching a herd of ewes for detecting those in estrus

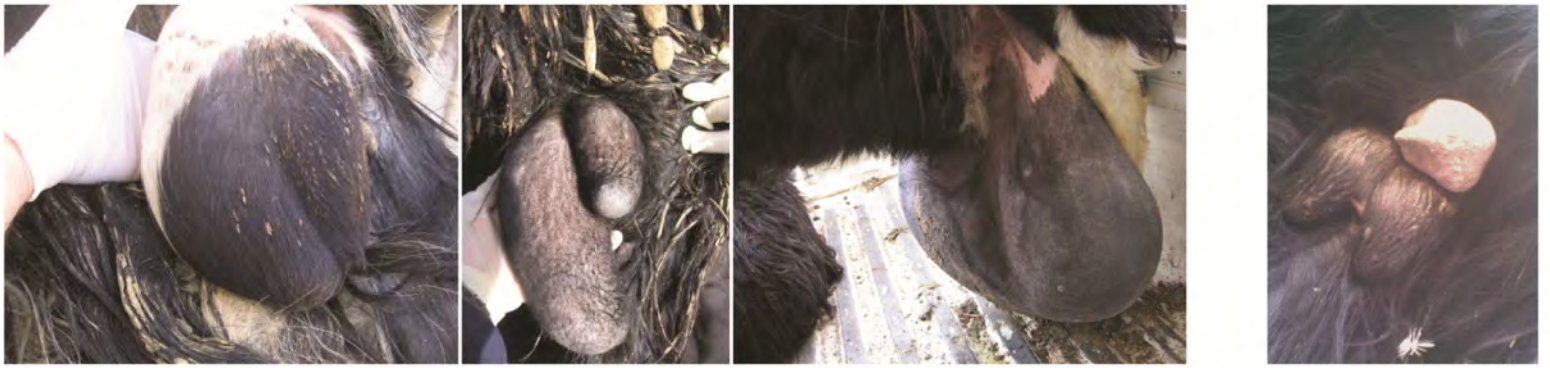
B) Hand mounting technique in sheep – need a help to hold the fatty tail on one side

Fig. 94: Sexual behavior and mating technique in rams (*Assiut-Egypt, 2005*).



A) Unilateral hypoplasia – right side, buck

B) Inflammation of the prepuce - buck



C) Unilateral orchitis (left) - ram

D) Unilateral orchitis (Left), buck

E) Bilateral orchitis - ram

F) Gynecomastia (enlargement of the udder in a ram)

Fig. B- 95: Diseases of the genital organs in rams and bucks (*Assiut-Egypt, 2003; Qassim-KSA, 20007-2013*).

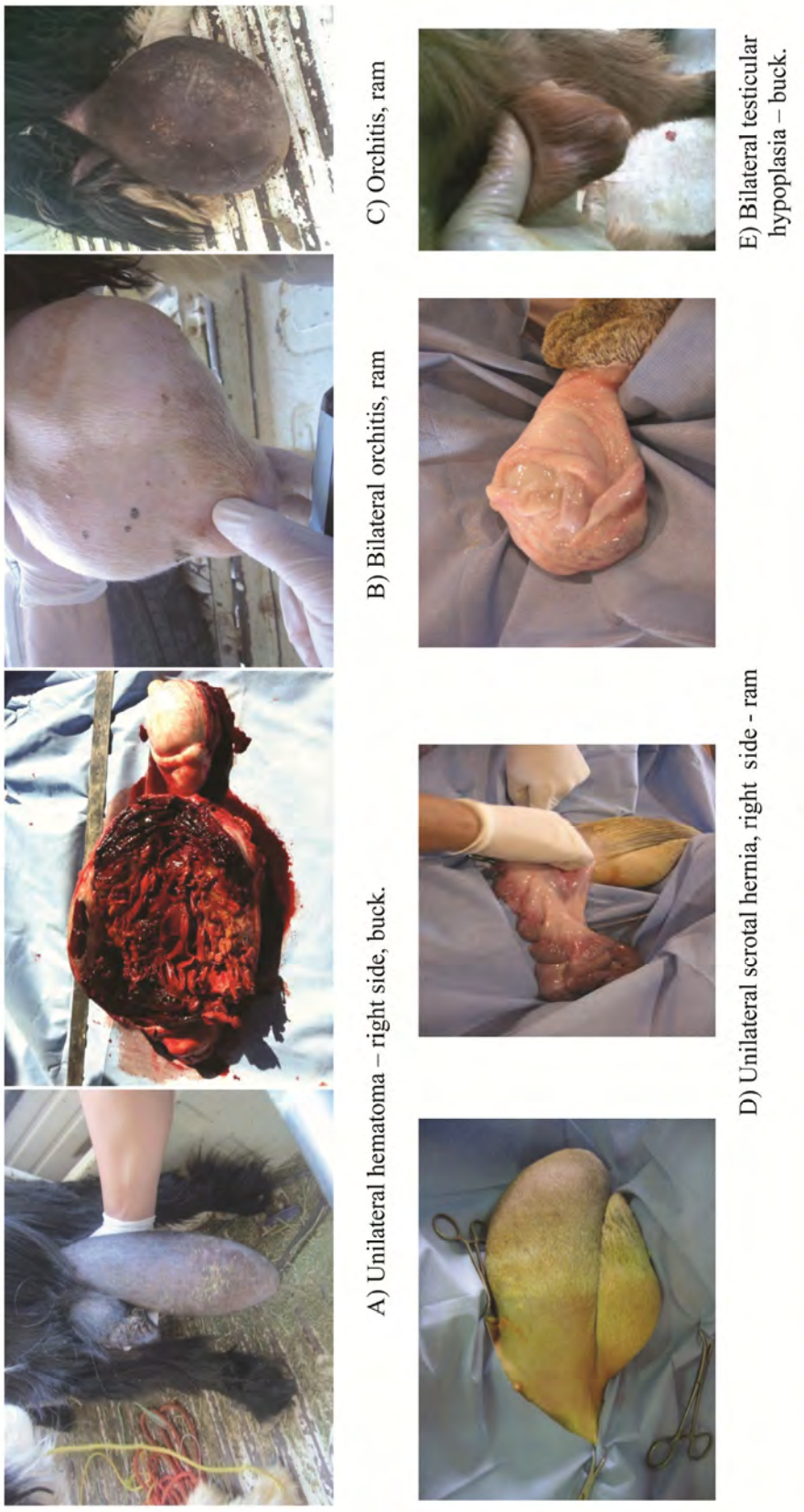


Fig. 96: B- Diseases of the genital organs in rams and bucks (*Qassim-KSA, 2007-2013*).

Chapter 12

Reproductive Technologies

Various techniques have been developed and refined to obtain a large number of offspring from genetically superior animals or obtain offspring from infertile (or subfertile) animals. These techniques include: artificial insemination, cryopreservation (freezing) of gametes or embryos, induction of multiple ovulations, embryo transfer, in vitro fertilization, cloning, etc.

Characters of ejaculate in farm animals are shown in Table (24) and some of the recent reproductive technologies applied in different animal species are illustrated in Figs. (97-108).

12.1. Artificial Insemination

Artificial insemination (AI) is the most powerful tool for livestock improvement ever available to the breeder. AI provides many benefits to the producer like: increase genetic gain by allowing increase selection pressure on the male population to hasten genetic improvement; increase offspring from a superior sire; availability of a superior sire to small flocks; frozen and fresh semen that can be shipped and stored and moved nationally or internationally with fewer health risks and welfare implications than live animals; disease control through testing of males before use and treatment of the semen with antibiotics to lessen the threat of disease; semen that can be frozen as insurance against injury or death of a superior sire; sire evaluations that can be carried out in many environments to assess the superiority of that sire over many different production types.

12.1.1. Artificial insemination in camels

Effective use of AI requires the dilution and storage of semen, but difficulties in semen collection and handling is impediment. The semen is highly viscous and as a result, assessment of sperm concentration, morphology, and motility, are difficult. Unlike the progressive motility of sperm seen in other domestic ruminants, only oscillatory movement is seen in the ejaculate. The role of high viscosity of camelids semen is not known, but it may act as a type of sperm reservoir or may be important for maintaining sperm viability within the uterus. To facilitate handling and processing of semen, attempts have been made to liquefy the ejaculate. In a study designed to test the effectiveness of enzymes for liquefying semen (i.e., collagenase, fibrinolysin, hyalurodinase, or trypsin), collagenase was effective in eliminating semen viscosity within 5 min, with little or no influence on sperm characteristics. All enzymes effectively reduced viscosity, but satisfactory motility was maintained only with trypsin or papain; i.e., collagenase was toxic at all concentrations. A mechanical

technique of liquefying the ejaculate involved alternately aspirating and expelling the ejaculate through a needle; this effectively liquefied the ejaculate and had little influence on other characteristics of semen.

12.1.1.1. Semen collection

Semen can be collected reasonably easy using an artificial vagina (AV) similar to that used for bulls with an inner rubber lining. The temperature inside the AV should be 40 - 41^{o}C. The water-jacketed semen collection flask should also be kept warm at 37^{o}C to avoid cold shock for the sperm. The female is then restrained in a sternal position, so that she cannot stand up when mounted, and the male is led up from behind. When the male sits down on the female and makes a few thrusts, the operator grasps the male's sheath and directs his penis into the AV and holds it there by manual pressure at the base of scrotum. The male will make several thrusts, interspersed by periods of rest, until ejaculation is completed and the whole process of semen collection, from intromission of the penis into the AV to the completion of ejaculation, can take an average of 5 minutes although it may occasionally take longer (20 - 25 min). Once collected, all further evaluation and handling of the semen should be carried out at room temperature and care must be taken to ensure it does not cool to below 20^{o}C without adding medium or extender. Each ejaculate must be carefully examined and pass a test for semen quality as well as meet rigid motility standards before it can be used for AI. The quality assessment includes, firstly, observing the general appearance of the semen, then measuring the volume, pH, motility and concentration as well as examining the morphology of the sperm. Semen collected from mature males trained to serve the artificial vagina should have at least 70% motile spermatozoa, but only the number of progressively motile spermatozoa is included in the calculation for the AI dose.

Electroejaculation can be applied using a custom-made electroejaculator and applying gradually increasing electrical stimulation. Electroejaculators are designed to stimulate the pelvic sympathetic and parasympathetic nerves with pulses of low voltage and amperage to induce penile erection and ejaculation. An electroejaculator set has the following components: carrying case, rectal probe, control unit, battery charger, power cord, probe cord, semen collection handle, collection cone and a collection vial. There are different kinds of electroejaculators available in the market. They may be battery operated or plugged into an electrical outlet. They may be manually operated, operate from a built-in program and may be programmable; some have all three options. Most bulls ejaculate with electrical stimuli of < 9 volts. There are also different kinds of rectal probes. All modern probes have three ventrally oriented electrodes.

12.1.1.2. Semen characteristics

Only two reports have been found regarding the chemical constituents of the seminal plasma of South American camelids—both on alpacas. The concentration of components such as chloride, calcium, total proteins, inorganic phosphate, and glucose, were similar to that in other ruminants, but citric acid and fructose concentrations were much lower than that in bulls, and horses-a feature thought to be consistent with the lack of vesicular glands in camelids. Although it is not yet clear whether the sugars act as an energy source for sperm metabolism or as signaling molecules to modulate sperm

function (e.g., capacitation), fructose and glucose are routinely incorporated into the majority of extenders for the semen of bulls and rams. The mean characteristics of camel's semen are:

- The semen is whitish or creamy in color, is odorless, but has gelatinous character and a pH of 7.37 $\pm$ 0.06.
- The volume of the ejaculate can be highly variable between individuals, but the average volume produced is 4.35 $\pm$ 1.86 ml (range 1.0 - 12.5 ml) with 70% of males producing below 7.0 ml.
- The average total number of spermatozoa per ejaculate is 24.32 $\pm$ 1.04 x 10^9 cells, giving a mean concentration of 5.59 $\pm$ 1.20 (range 2.2 - 12.5) x10^9 sperm/ml.
- On average the total number of motile sperm per ejaculate is 23.10 x 10^9 sperm with generally 70 - 90% being progressively motile, 5.01% $\pm$ 1.52 on average being dead and 4.9% (2.2 - 6.5%) being abnormal.
- The length of the sperm is 51.05 $\pm$ 4.54 μm, the length of the head is 7.68 $\pm$ 1.64 um.

12.1.1.3. Insemination

Dilution of freshly collected semen

Several extenders have been used for dilution of freshly collected camel semen such as skimmed milk-glucose extender, Dimitropolous 11, Laiciphos, Androhep, glucose–EDTA, sodium–citrate-egg yolk and lactose-egg yolk, and Green buffer egg yolk. Most of these extenders contain an energy source, a protein for cold shock protection (lipoprotein from egg yolk or casein from milk), a buffering system and antibiotics. Once collected the semen is diluted in a ratio of 1:1–3:1 (extender:semen) depending on the concentration of the ejaculate, with warmed (30–35 ◦C) extender added slowly to the semen. It is better to then allow the semen to liquefy before insemination to aid better mixing of the semen with the extender and to allow more accurate assessments of concentration and motility. More recently, further studies have compared the extender INRA-96 with Green Buffer. Results showed that whereas motility was higher after dilution in Green Buffer (67%) compared with INRA-96 (59%), membrane integrity was higher after dilution in INRA-96 (65%) compared with Green Buffer (56%). However, sperm viability and acrosome integrity were similar for both buffers and pregnancy rates were unaffected by diluent, Green Buffer (34%) and INRA-96.

Dilution of semen in the above extenders and keeping at room temperature or 37 ◦C is only suitable if the semen is to be inseminated within an hour or so of collection. For longer preservation in the liquid form (up to 48 h) semen needs to be cooled slowly to 4–5 ◦C. Slow cooling can be achieved by putting the tube of extended semen into a beaker of water at room temperature and placing in the refrigerator which allows it to cool to 5 ◦C over 1 h. Alternatively the semen can be cooled in an equine Equitainer where the cans used to cool the equitainer are placed in a freezer for at least 24 h prior to use. The semen is then sealed in a plastic universal, wrapped in two thermal blast bags at room temperature and placed within a plastic cup inside the equitainer before closing the lid. The advantage

of using an equitainer is that semen can be shipped between farms, or even countries, providing it can be inseminated within 24 h and has a motility of at least 35–40%. Studies have reported pregnancy rates of 25–30% after insemination of chilled semen diluted in Green buffer + 20% egg yolk but, this is much less than the 50–55% pregnancy rate reported in camels inseminated with fresh, diluted semen. In a more recent investigation the authors compared Green buffer with INRA-96 for chilling camel semen, and found that whereas motility, membrane integrity and acrosome integrity were similar, sperm viability was higher after chilling in INRA compared with Green Buffer. In comparison with the earlier study their pregnancy rates were higher after AI with semen chilled in INRA (17.6%) than with Green Buffer (0%).

Deep freezing of camel semen

This technique uses two extenders, a cooling extender (11% lactose + 20% egg yolk), which is added immediately after collection, and a freezing extender (cooling extender + 6% glycerol + 1.5% OEP-Equex paste), which contains the cryoprotectant glycerol and an emulsifying agent (OEP paste) to help stabilize sperm plasma membranes.

The method used for diluting and freezing the semen was as follows:

Dilution and cooling of straws:

- Diluted semen at 25–30 ◦C (1:1, v:v) with cooling diluents (11% lactose + 20% egg yolk)

- Cooled to 15 ◦C in 2.5 h.

- A second dilution with cooling diluent to give a sperm

concentration of 150× 106 ml–1

- Cooled to 5 ◦C in 1.5 h. - A further dilution to sperm concentration of

100× 106 ml–1 with the above cooling diluent containing

6% glycerol and 1.5% OEP (Equex) giving a final

glycerol concentration of approximately 2%.

- Filled 0.25 ml/4 ml straws (Fa. Makrotub, Landshut, Germany) with diluted semen.

Freezing of straws:

- from 58 ◦C to −120 ◦C by suspending in liquid nitrogen vapour for 20 min;

- then plunged into liquid nitrogen (−120 ◦C to −196 ◦C).

Thawing semen

Carried out in a water bath

- 0.25 ml straws: 40 ◦C for 10 s,

- 4 ml straws 50 ◦C for 40 s.

Semen deposition

Either rectal palpation or teasing is used to detect females at the correct stage of their follicular wave cycle to be inseminated. If rectal palpation is used, the ovarian follicles should be no less than 12 mm in diameter and if the females are teased by males, they should be inseminated five days after first showing sexual receptivity. Females are inseminated in a sternal position in a comfortable, clean place as an atmosphere of calmness is required. The rectum is evacuated of feces and the perineal regions are cleaned thoroughly taking care to ensure no feces are wiped between the vulva lips. Insemination is carried out using an insemination catheter that is connected to a pre-warmed syringe containing the thawed semen. Then with a gloved hand in the rectum holding the cervix, the operator directs the inseminating catheter through the vagina and into the external os of the cervix so that the semen is deposited at the anterior end of the vagina or in the body of the cervix. Tran-vaginal insemination may also be practiced. Many others preferred to inseminate the camels per vagina, as the camel cervix is difficult to get hold. In this case, the operator guiding the AI pipette through the cervix with the hand in the vagina.

Using this technique they achieved post-thaw motility rates of 70% but, 48% were morphologically abnormal o result was recorded regarding the 0.25 vs. 4 ml straw packaging method and the semen was not inseminated.

Fertility traits with frozen-thawed semen

The following studies were performed to evaluate the fertility results for frozen-thawed camel semen. Pregnancies were diagnosed by measuring progesterone and confirmed by recording the calving. The treatment protocols were as follows:

1. Double insemination: females were inseminated twice 24 hours apart with two ampoules of frozen semen. Each ampoule contained 0.5 ml native semen (diluted to 2.0 ml with extender) with 2.975×10^9 motile spermatozoa and 1.48×10^9 progressively motile spermatozoa. The first insemination was used for inducing ovulation and the second for pregnancy. Fertility was 95.77%.

2. hCG injection/single insemination: 1000 iu hCG was injected intravenously (i.v.) into each female to induce ovulation, and two ampoules of frozen semen were inseminated 24 hours later. All ten inseminated camels conceived (100%).

3. LH injection/single AI: 200 iu LH was injected i.v. into each female, and two ampoules of frozen semen were inseminated 24 hours later. Again all ten inseminated camels conceived (100%).

4. Single AI/double dosage: Females were inseminated once with four ampoules of frozen semen. All ten inseminated camels became pregnant (100%).

5. Single AI/single dosage: Females were inseminated with two ampoules of frozen semen. Four of five inseminated camels were pregnant (80%).

6. Single AI with 10 times diluted semen with GnRH supplemented extender SYG-2. Each ampoule contained 2.0 ml diluted semen and 100ug GnRH analogue. Two ampoules were inseminated and therefore the total dosage of GnRH inseminated intravaginally into each camel was 200ug, the total sperm number was 5.6×10^8 with 2.9×10^8 being progressively motile spermatozoa. All of the 45 inseminated camels were diagnosed pregnant (100%).

7. Single AI with 15 times diluted semen with GnRH supplemented extender SYG-2: The dosage of GnRH and the volume of semen inseminated were the same as in Group 6, but the total number of spermatozoa was reduced to 3.6×10^8. Forty-eight out of forty-nine inseminated camels were confirmed pregnant (98%).

Extenders used for llamas and alpacas have ranged from the simplest, such as sodium citrate in combination with egg–yolk, a sugar or skim milk, to a more complex media containing phosphate buffered saline, bovine serum albumin, and inorganic/organic buffers such as Tris and Hepes in combination with various sugars (lactose, fructose or glucose).

Sperm collected from the caudal epididymis has been used to avoid the complications of the viscous ejaculate, and for use in IVF. Spermatozoa from llama epididymides submitted to slow refrigeration to 5 ^{o}C after dilution with Kenney, Tris–yolk or Colorado semen extender, maintained a progressive motility of 50, 30 and 20%, respectively after 72 h. Epididymal alpaca spermatozoa diluted with a lactose-based extender and frozen in pellets had the highest percentage of motility and intact acrosome morphology.

Ejaculates collected from two llamas and three alpacas by artificial vagina were liquefied with collagenase (1 mg/mL) and diluted first with a mixture of sodium citrate (2.9%) and egg yolk (10%) and then with 7% glycerol added at 15 min intervals in three equal parts prior to freezing. Sperm motility was estimated at 80% before freezing, 60% after final dilution and cooling (before freezing), and 30–40% after thawing. Ethylene glycol was used as a cryoprotectant for alpaca semen collected by AV, liquefied by mechanical action, and diluted with skim milk and fructose. Post-thaw motility was 30% and highly correlated with estimates of live sperm and the acrosome reaction. Most studies have reported that sperm motility ranging from 15 to 35% after thawing. In a report of the first live births of South American camelids after AI with frozen semen, the semen of alpacas and llamas was treated with 1 mg/mL of collagenase, followed by a two-step dilution in sodium citrate–egg yolk extender, and frozen over liquid nitrogen vapor. After thawing, sperm motility ranged from 40 to 50%, and 5/19 inseminations resulted in live births. The inclusion of dimethyl sulfoxide (DMSO) as a cryoprotectant had a negative effect on sperm motility and viability of llama semen.

Problems with AI in camels

Results of AI in dromedary have been less encouraging. Workers at the Camel Reproduction Centre, Dubai have claimed pregnancy rates of 50-60% with fresh diluted semen used within 30 minutes of collection, but conception rate decreased dramatically to 25-30% if semen was not used for 24 hours. All the pregnancies were established with a particular extender and no pregnancy could be established with frozen thawed semen. Likewise, pregnancy results from almost nil with diluted-

chilled/frozen-thawed semen to 40% with whole semen have been reported in India. The reasons for poor success have not been determined but a major difficulty with AI in camel is to ensure ovulation in inseminated animals. The incidence of ovulation and pregnancy is significantly lower in female camels inseminated either with fresh undiluted or diluted semen alone than those obtained with AI following mating with vasectomized teaser. Deposition of 1 ml of fresh semen, or else exogenous administration of hCG, is essential to ensure ovulation. Ovulation in inseminated camels can be regularly achieved with a single injection of GnRH given 34h before or at the time of insemination. Entrapment of spermatozoa in thick viscid camel semen and its speculated role as a sperm reservoir in the female genital organs also need to be considered. Best liquefaction and progressive sperm motility is achieved in Tris–lactose egg yolk extender.

12.1.2. Artificial insemination in horses

12.1.2.1. Semen collection

The stallion when collected with a properly prepared artificial vagina will follow a similar pattern of thrusting to that of natural mating. To ejaculate into an AV it is not necessary for the stallion to jump a mare or a dummy. Once the stallion has achieved a full erection, his penis is washed with clean water, dried and inserted into a properly prepared AV. The stallion in many instances will vocalize and move forward until the pelvic thrusts start at which time he might lift his front legs slightly or arch his back and have all four limbs on the ground until ejaculation. Several models of AVs are available on the market, and although each has advantages and disadvantages, each operator should choose a model based on stallion suitability and ease of use. All AVs work under the same principle, which consists of a double rubber liner, usually latex, that will provide space to form a water jacket that at the adequate temperature and pressure will stimulate the stallion to ejaculate. The temperature in the water jacket to stimulate a stallion to ejaculate is at between 42 and 4ȝC. Furthermore, proper lubrication with a nonspermicidal water-soluble lubricant and the proper pressure are critical for adequate stallion stimulation. Condoms provide an alternative for obtaining a semen sample, particularly from stallions that are reluctant to ejaculate in an AV.

12.1.2.2. Semen handling

Raw semen is very fragile and should not be exposed to direct sunlight or sudden temperature changes. Once the stallion ejaculates, the semen should come into contact with a clean and warm container to prevent cold shock. The semen is filtered in line during ejaculation or filtered immediately after collection with nylon mesh or milk filter paper. This procedure is done to remove all extraneous particles and the gel fraction. Immediately after collection the raw semen should be placed in an incubator at 37 ^{o}C prior to extension. The volume and color of the ejaculate should be recorded. In addition, the other physical characteristics of the ejaculate such as sperm movement, sperm concentration, and sperm morphology should be assessed from a small aliquot (1–2 ml) of raw semen that is removed prior to the addition of any diluents or extender. Once semen is diluted with a pre-warmed extender it should be immediately removed from the incubator and cooled down to room temperature (20°C). An accurate assessment of the percentage of progressively motile sperm can only be done when the semen has been diluted to 25 million to 50 million sperm per milliliter.

12.1.2.3. Semen preservation

It is recommended to always place the semen in an appropriate extender even if it is going to be inseminated within a few minutes. A proper extender buffers pH changes of the raw semen, maintains the osmolality, provides energy and protein sources for sperm metabolism and membrane stabilization during thermal changes, reduces the detrimental effects of the seminal plasma, and provides antibacterial properties through the antibiotics. Semen can be preserved for varying periods of time. However, the state for more than 12 hours in a Kenney type extender is between 4 and 6 ^{o}C. There are several containers currently available commercially that are designed for cooling and transport of equine semen. The Equitainer is the most widely used container for passive cooling and transporting equine semen.

12.1.2.4. Semen extender and dilution rates

The majority of extenders used for fresh and cooled equine semen are nonfat dried milk solids with glucose and antibiotics. An ionic extender supplemented with phosphocaseinate is also used. On the other hand, stallion semen could be centrifuged and the sperm is re-suspended in an egg yolk based extender for fresh or cool shipping. The most common antibiotics include a combination of potassium penicillin and amikacin sulfate, gentocin, and ticarcillin.

The ideal dilution rate at which sperm should be extended will vary according to the sperm concentration in the raw semen and the total number of progressively motile sperm. Semen with 100 million sperm should be diluted at a 1:3 ratio (semen: extender). When the concentration of the ejaculate is between 450 million and 500 million per milliliter the dilution rate would be 1:10. Extending the semen using this method will result in a final sperm concentration of 25 million to 50 million per milliliter. The final volume to be shipped will be calculated so that a total of at least 1 billion sperm cells are sent to the mare. The packaging of the semen when using the skim milk glucose extender should be performed under anaerobic conditions. Semen can be packaged in preloaded syringes, disposable baby bottle liners or whirl pack bags, provided that all the air is removed from the package prior to placing it in the container.

12.1.2.5. Insemination of cooled semen

If the semen is of good quality and the mare is a young fertile mare, she could be inseminated once or twice at 12- or 24-hour intervals. However, if the semen has poor quality it would be important to breed the mare as close to ovulation with the entire dose. On the other hand, if the semen is of good quality but the mare is a problem mare with a tendency to accumulate uterine fluid, she should be bred only once within 24 hours prior to ovulation.

12.1.2.6. Frozen semen

In general, the freezing process involves the collection of semen from the stallion, evaluation of the semen, dilution and centrifugation, and resuspension of the sperm in freezing extender. Unfortunately frozen-thawed sperm appear to have a shorter life than fresh or cooled sperm. Owing to apparent short lifespan of cryopreserved semen the insemination process with frozen semen requires

more infrastructures and is more labor intensive than insemination with fresh semen and often results in reduced fertility compared to other breeding methods.

12.1.2.7. Facilities

Because it is imperative to breed the mares close to ovulation, accuracy in the prediction of the time of ovulation is critical. Therefore, the availability of ultrasound equipment is required. In addition, it is strongly suggested that the facility where the mares are inseminated be equipped with a liquid nitrogen tank for the storage of semen. Thawing, evaluating, and loading the semen requires an incubator, an accurate thermometer, a clean water bath, a good quality microscope, clean slides, coverslips, and a watch.

12.1.2.8. Pregnancy rates with frozen semen

Straws of 0.25 or 0.5ml are generally thawed at 37 ^{o}C for a minimum of 30 seconds. Care must be taken if multiple 0.5 ml straws are thawed at once to ensure that the straws will not adhere to each other while thawing, which will create an uneven thawing rate in the straws.

Sperm that have been frozen and thawed appear to have reduced longevity because of the membrane changes inflicted on them during the process of cryopreservation. Therefore, to maximize fertility, it becomes critical to try to breed the mares as close to ovulation as possible. Higher pregnancy rate could be achieved when insemination is performed within 6 to 12 hours prior to ovulation. If mares have not ovulated within the first 12 hours after the AI, then a second dose should be inseminated immediately after ovulation has been detected on rectal examinations performed every 12 hours. Fertility of mares that are bred only once after ovulation when rectal examinations are only performed every 12 hours is reduced by an additional 20% compared to those mares bred only once before or before and after ovulation. An ovulatory inducing agent can be used in order to reduce the number of examinations and to reduce the time from breeding to ovulation. Two products are currently available to induce ovulation in mares: human chorionic gonadotropin (hCG) and GnRH analogue. Typically 2500 IU of hCG are given intravenously when the mare is displaying behavioral estrus, has a follicle of at least 35mm, and has obvious and distinct edema of the endometrial folds. If the same criteria are followed to treat mares, GnRH induces a reliable ovulation in a narrower window (38 hours) after treatment.

In general multiple (4–8) 0.5- or 0.25-ml straws are necessary for one dose and should be thawed at 37 ^{o}C for at least 30 seconds. The semen should be evaluated microscopically prior to inseminating the mare.

The post-insemination examination is a crucial component of the insemination process and should be done not more than 12 hours after insemination. The purpose of this examination is to confirm that the mare has ovulated if she was bred prior to ovulation and to determine the presence of inflammatory exudates or fluid accumulations in the uterus regardless of the time of breeding. This is particularly important in old maidens, mares with delayed uterine clearance, and mares susceptible to uterine infections. Appropriate therapies such as uterine lavage, oxytocin injections, post-breeding antibiotic infusions, and Caslick's procedures should be performed.

12.1.3. Artificial insemination in cattle

12.1.3.1. Semen collection

The AV is the optimum method for semen collection in bulls. The AV temperature of 42 is optimum for obtaining ejaculates of optimum quality. Bulls to be collected by AV must be halter broken and should be nose ring trained. Since semen collection by AV imitates natural breeding, the bull must mount a cow in heat, a steer, a dummy cow, or a bull while the collection is being performed. When the bull has mounted, the collector must grasp the penis through the sheath and direct it to the opening of the lubricated AV. The bull will make seeking motions and thrust into the AV. The thrust should be vigorous to ensure an ejaculate has been collected.

Semen can be collected by transrectal massage: this technique requires two people, one to do the massage and one to collect the semen. The bull is held in a chute. After removal of feces from the rectum, a longitudinal back and forth massage is applied mainly over the ampullae, drawing semen toward the pelvic urethra. When the urethral muscle begins to pulsate the massaging action should be in synchrony with the pulsations. The semen collector must collect the cloudy fluid into a warm receptacle as it dribbles from the penis or prepuce. The extended penis may be held by the semen collector during rectal massage to facilitate collection of a clean semen sample.

Semen can also be collected by electroejaculation. To obtain the best results with each individual bull it is important to choose the right probe size since its weight and diameter influence transmission of the stimulus to the nerves. Probes with larger diameter produce a stronger response to stimuli of a given electrical output than smaller diameter probes. The recommended probe diameter for bulls weighing 550 - 900 kg is 6.5 to 7.5 cm. For larger bulls, a 9 cm diameter probe may be necessary to achieve ejaculation. Bulls are restrained in a chute with good footing without the head caught in a head gate. It is extremely important to locate at least one strong pole behind the bull. With only one restraining pole, a height of 71 - 76 cm may be preferred if the scrotum and testicles are to be examined. A lubricated probe is introduced into the rectum with the electrodes facing ventrally. Make sure the electroejaculator is turned on before performing this step. If it is turned on after inserting the rectal probe, the bull may receive a strong electrical pulse that will increase the level of stress in the animal. Electrical stimulation is begun slowly until the bull shows a minimal response. Consecutive stimuli are then given, each increasing in intensity a small amount. Stimuli should last 1 - 2 seconds and then be discontinued for 0.5 – 1 second before the next one starts. After several stimulations, clear pre-seminal fluid begins to flow from the protruded penis. This clear pre-seminal fraction should not be collected. As soon as the cloudy sperm rich fraction begins to flow from the penis, a collection cone with the test tube is placed over the penis and the sample is collected. After collecting a suitable sample, the stimulation is stopped and the rectal probe is removed. The semen sample is then taken to the lab for evaluation and processing. While performing the procedure is important to obtain penile protrusion for examination of the penis and prepuce. The majority of the bulls emit semen without excessive stimulation. However, if a bull has not ejaculated after reaching the highest level of stimulation, 3 - 4 stimulations in the maximum level can be done, followed by a rest period of 1 - 2 minutes with the probe still inside the rectum. Often on a second attempt to electroejaculate bulls, the penis will not protrude; therefore, while the bull rests, the penis should be held manually with a gauze

sponge to prevent retraction into the preputial cavity. All four fingers with the gauze should wrap the glans penis. Do not attempt to catch the penis over the preputial region, as it will roll off into the prepuce. Semen emission commonly occurs during the rest period thus the collector should be attentive to catch the emission. After resting the bull, stimuli beginning at two voltage increments below the maximum are begun in a second attempt to obtain an ejaculate. This is often successful. Bulls should be sexually rested for 1 - 2 days before electroejaculation to allow sperm to accumulate. With good equipment and proper technique; only about 2% of normal fertile bulls fail to emit semen by electroejaculation. Electroejaculation without anesthesia is considered to be painful to bulls and is controversial in some countries. Vocalization during electroejaculation and elevation of circulating adrenal progesterone and cortisol after electroejaculation are evidence of pain. Therefore, the procedure must only be done by personnel with proper training and always in the gentlest way possible.

12.1.3.2. Semen characteristics

The average volume for a mature bull is 4-5 mL (range 1-11 mL). Density may be classified as follows: Very Good (ddd): creamy, grainy semen with 750 to 1 billion or more spermatozoa per ml; Good (dd): milk-like semen with 400 to 750 million spermatozoa per ml; Fair (d): skim milk-like semen with 250 to 400 million spermatozoa per ml; Poor (-): translucent semen with less than 250 million spermatozoa per ml. Semen collected by AV may be more concentrated, and cleaner, than samples collected by electroejaculation or by massage.

To estimate gross motility, a 5 mm diameter drop of the semen is placed on a warm glass-slide and mass motion is observed under bright field microscopy at 10 X magnification with the field diaphragm closed. Descriptive assessment of gross motility: Very Good (+++): rapid dark swirls and eddies; Good (++): slower swirls and eddies; Fair (+): no swirls, but prominent individual cell motion; Poor (-): little or no wave motion.

To estimate individual motility, a 5 - 7 µl volume of the semen is placed on a new warm glass-slide creating a drop approximately 3-5 mm in diameter, which is then covered with a coverslip. The volume of semen used (5 vs. 7 µl) for evaluation will depend on the size of the coverslip preferred (18x18 mm or 22x22 mm coverslips). The sample is observed under phase contrast microscopy at 200 - 400 x magnification and the percentage of sperm cells having progressive linear motion is determined. If the semen is too concentrated, the semen sample may be diluted with a buffered diluent or semen extender before coverslipping. Visual microscopic analysis of individual progressive motility is somewhat subjective even when performed by very skilled people and becomes tedious when large numbers of samples must be analyzed. Computer Assisted Semen Analysis (CASA) systems have the potential to increase objectivity of analysis and reduce worker fatigue. In addition, CASA systems should reduce variability in analytical. For good sample >75% individual motility is acceptable.

To evaluate sperm morphology phase contrast microscopy or the use of sperm stains are needed. Eosin-nigrosin stain is commonly used as a "live/dead" stain because in addition to providing background-contrast for sperm cells with the nigrosin component, sperm membrane penetration by eosin, or lack thereof, is an indicator of sperm membrane integrity and thus of sperm viability.

Technique: put a glass slide on a warming plate (37 °C) for 30 - 60 seconds. Put a 5 - 6 mm droplet of eosin-nigrosin stain at one end of the glass slide. Put a droplet of semen beside the droplet of stain. The droplet's size depends on the density of the semen. Mix the stain and the semen on the slide. Spread the mixture slowly on the slide from one end to the other using a wooden applicator stick or the edge of another glass slide. Dry the smear quickly by blowing air over it. Perform the sperm morphology evaluation at 1000 x magnification using immersion oil, counting at least 100 sperm per sample. If a high number of abnormalities are observed, a count of 300 or more sperm will give a more accurate differential count. Eosin-nigrosin stain is very hypotonic and, therefore, may cause artifacts in sperm morphology. Sperm abnormalities should not exceed 15% for a good sample.

For assessment of sperm concentration the following methods can be used to determine sperm concentration. Hemacytometers are used as the standard method for determination of cell concentrations and for calibration of electronic systems of cell counting. The method is very reliable and inexpensive, but will take about 10 minutes per sample and involves somewhat tedious visual counting of sperm. Sperm dilution prior to filling the 2 chambers of the hemacytometer can be done in a variety of ways. CASA Systems such as SpermVision (use computer programmed digital analysis of microscope fields of moving sperm) can be used. Several characteristics of motility are quantified, and sperm concentration is also determined. These systems require appropriate dilution of semen samples before filling of one or more commercially supplied disposable counting chambers with a depth of 20 microns. A counting chamber is placed under a microscope and up to 7 microscope fields are analyzed in just seconds per field. Data can be stored and provided on customized printed forms. The average sperm concentration for a mature bull is 1.00.0000 sp/mm3 (range 800.000-1.200.000 sp/mm3).

12.1.3.4. Storage of frozen semen

Maintenance of low temperatures is important to the successful storage of frozen semen. Care should be taken especially for the upper third of the neck of the tank where canes and goblets are raised for removal. When straws are exposed to these temperatures the semen temperature rises quickly. The thermal response of semen in 0.5-ml straws exposed to temperatures of −22°C (2 inches from the top of the tank) and 5 °C (1 inch from the top of the tank) is shown in Figure 34-2. The time required to reach −100 °C to −80 °C, which is the beginning of ice recrystallization, is approximately 10 to 12 seconds for both temperatures. Thermal injury to sperm is permanent and cannot be corrected by returning semen to the liquid nitrogen. For optimal maintenance of sperm viability, canisters and canes containing semen should be raised into the neck of the tank only for the time required to retrieve a single straw. This time should not exceed 5 to 8 seconds.

12.1.3.5. Semen-thawing procedures

The recommendation for thawing of semen frozen in straws is not the same for all AI organizations. For optimal results, the recommendations of the semen processor should be followed: warm-water thawing of straws for periods ranging from 10 to 60 seconds, the straw should be immersed in 30 to 35 °C water for a minimum of 40 seconds. Based on the scientific literature, warm-water thawing of semen at 35 to 37 °C seems most appropriate and safest for vaporfrozen semen packaged in 0.5-ml straws. As many as 10 straws can be thawed simultaneously in a thermostatically

controlled thawing bath without significantly decreasing sperm viability. Of note, sperm that remain in a non-thermostatically controlled bath tend to cool over time; this finding may explain the slightly better sperm viability in comparison with that of semen thawed with a thermostatically controlled bath, because the metabolism of cells is lower in the non-thermostatically controlled bath. Thermostatically controlled water baths are designed to maintain a temperature of approximately 35 ^{o}C. Regardless of the number of straws thawed simultaneously, straws should be agitated immediately after being plunged into the water, to prevent them from freezing together during the thaw. Duration of incubation time in the bath should be minimized but also depends on the ambient temperature. Ambient temperatures below 20 ^{o}C dictate that straws remain in the bath until immediate insemination is possible. By contrast, high ambient temperatures allow the immediate removal of straws once thawed; thus, holding time occurs in the insemination rod.

12.1.3.6. Semen handling after thawing

Regardless of what type of water bath is used, all water should be thoroughly removed from the straw before it is cut. Osmotically, exposure of the 0.5-ml dose of semen to as little as one drop of water results in irreversible cell injury. Rarely, a straw may be defective and leak, permitting water to enter during thawing. If this is suspected, that straw should not be used. A major concern with warm-water thawing is the danger of cold shock caused by mishandling of the semen after thawing.

Cold shock is the irreversible injury to sperm caused by a rapid decrease in semen temperature above freezing after thawing. Cold shock occurs when semen is thawed and then subjected to cold environmental temperatures before insemination. The severity of damage depends on rate and span of temperature drop. It results in loss of motility, metabolic activity, and fertilizing potential and is believed to involve irreversible changes in the outer plasma membrane.

Cold shock occurs most frequently when breeding is undertaken in cold weather and particularly when warm water thawing is used. As might be expected, the high surface-to-volume ratio of the straw makes it vulnerable to cold shock. In cold weather, either of the following precautions seems warranted: (1) provide a sheltered heated area for breeding, or (2) provide a sheltered heated area for semen thawing and loading of the insemination pipette near the animals to be bred; then insulate the inseminating equipment and carry it to the breeding chute. Freezing weather brings the possibility of refreezing thawed semen. This can be classified as a disaster with regard to semen quality. Regardless of the thaw procedure recommended, conditions causing a sudden drop in the temperature of the semen should be avoided.

12.1.3.7. Semen thawing and handling tips

It is essential that frozen semen be handled and thawed carefully and properly to maintain sperm viability for optimal results. Insemination equipment should always be kept clean, dry, and warm. A thermometer should be used to obtain the proper water temperature. The thermometer should be checked for accuracy at least every 6 months with a reference mercury thermometer. When removing the straw from the nitrogen tank, the handler should gently shake the straw to remove any liquid nitrogen that may be retained in the cotton plug end of the straw. The thaw should be timed with

a watch to avoid guessing. While the semen is thawing, the handler warms the insemination rod by rubbing it briskly with a paper towel. Once it has been warmed, the handler places the insemination rod within his or her clothing so that it will be close to the body to maintain warmth. After the semen is thawed, the straw is dried thoroughly with a paper towel and protected from rapid cooling. The air space in the straw should be adjusted to ensure that no semen will be lost when the end of the straw is cut off. This can be done by slightly flicking the wrist while holding the straw at the crimp-sealed end. Only sharp scissors or a specially designed straw cutter should be used to cut the straw. The straw is cut "square" at a 90-degree angle to achieve a good seal with the sheath. The assembled insemination rod is wrapped in a clean, dry paper towel and tucked within clothing for transport to the cow. Finally, the cow should be inseminated within minutes after the semen has been thawed. The period of time between removing the straw from the tank and depositing the semen in the cow should not exceed 15 minutes.

12.1.3.8. Semen deposition

Practice is required to develop the skill, which should be learned and periodically reviewed with the assistance of professionals. One of the most critical parts of the insemination technique is depositing the semen anterior to the cervix. A majority of sperm deposited by natural service is lost from the reproductive organs shortly after being deposited. The major reason why sperm numbers can be markedly lower in each dose of semen used in AI is that the cervix, which is the major barrier to sperm transport, is bypassed in correct semen deposition with AI. A significant increase in fertility has been reported when bilateral cornual insemination was performed. Others reported improved fertility when cornual insemination was performed. A tendency for improvement was observed, however, when inseminators who were achieving low conception rates by attempting to deposit semen in the uterine body were retrained to deposit semen in both uterine horns.

12.1.4. Artificial insemination in Buffaloes

12.1.4.1. Techniques of semen collection

These resemble that described for cow-bulls.

12.1.4.2. Semen characteristics

Buffalo semen is milky white; never yellow. Its consistency depends on its content of spermatozoa and is affected, among other factors, by frequency of ejaculation. Sperm concentration is about 800 million /ml on the average. Values - as high as 1500-2000 millions sperm/ml and as low as 200 million sperm /ml have been recorded. First ejaculates contain higher number of spermatozoa per ml compared to second ones. The percentage of abnormal spermatozoa in buffalo semen varies between 3 and 26%. Buffalo spermatozoa have distinct morphological features. A typical spermatozoon is shorter than that of *Bos taurus* bulls and measures 62 microns, on the average. The mean head length, maximum head breadth and breadth at the base of the head are reported to be 7.20, 4.45, 2.40 microns, respectively. Head area is between 24.3 and 26.6 sq.micron and head shape index

1.62. Various mensuration characteristics of buffalo spermatozoa are affected by seasons, feeding regime and sequence of ejaculation. The semen characteristics were studied in 182 ejaculates collected with a bovine artificial vagina from five swamp buffalo (*Bubalus bubalis*) bulls. The mean values were: volume, 2.9 ml; general motility, 70.7%; live (unstained) sperm, 86.5%; abnormal sperm, 10.3%; intact acrosomes, 82.4%; sperm concentration, 1.06×10^9 sperms/ml and total sperm/ejaculate, 3.18×10^9 sperms/ml. Among the sperm abnormalities noted were "knobbed" acrosome, abaxial implantation, the "Dag" defect and the corkscrew midpiece.

12.1.4.3. Biochemical characteristics

Buffalo semen contains higher fructose, acid and alkaline phosphatase activity and inorganic phosphorus, but lower ascorbic acid concentration than bull semen. Acid phosphatase shows significant direct correlation with sperm concentration, percentages of initially motile and live spermatozoa, dehydrogenase activity and rate of fructose utilization. On the other hand, and in contrast to bull semen higher alkaline phosphatase in buffalo semen is concomitant with decreased motility, per cent live cells, depressed dehydrogenase activity and a slight decrease in fructolytic rate. It could be inferred that higher concentration of alkaline phosphatase in buffalo semen influences adversely the viability of spermatozoa during *in vitro* storage. Lower levels of ascorbic acid in buffalo semen may be responsible for poor *in vitro* preservability of buffalo spermatozoa since it has been demonstrated that buffalo spermatozoa are more susceptible to oxidative damage during storage and preserve better under a reduced environment. Both acetylcholinesterase and amylase are lower than in bull semen. Buffalo semen contains characteristically low levels of potassium compared to sodium. The sodium: potassium ratio in buffalo seminal plasma is 1:3.7 on the average with well over 60% of the ejaculates having a ratio of 1:2.86. A difference exists between the nucleic acid contents of bull (3.52 pg /sperm) and buffalo bull (3.25 pg /sperm) spermatozoa. Total protein content in buffalo (2.27 g /100ml) is also significantly less than in bull (5.43 g /100 ml) semen. As in bulls, glutamic acid is most predominant in seminal plasma but arginine is predominant in spermatozoa. The DNA / arginine ratio is significantly higher in buffalo (1.08) than in bull (0.93) spermatozoa. There are also considerable qualitative differences in buffalo seminal protein fractions separated by starch gel electrophoresis. Fructose utilization rates and fructolytic indexes are significantly higher in semen of buffalo bulls compared to that of *Bos taurus* bulls. Partial correlation studies revealed that fructose consumption in buffalo semen is primarily influenced by sperm cell concentration. Initial fructose level does influence fructose utilization acting as a rate-limiting factor. Moreover, the live sperm per cent in buffalo semen independent of initial fructose level, sperm concentration and initial motility influences considerably the rate of fructolysis, whereas initial motility independent of other factors does not correlate with the amounts of fructose utilized.

Buffalo sperm acrosome is rich in hydrolytic enzymes, as alkaline phosphatase and beta-glucuronidase, but acid phosphatase is localized mainly in the post- acrosomal segment.

12.1.4.4. Preservation of buffalo semen

The conventional egg-yolk citrate extender originally developed for bull semen appears not so suitable for cold preservation of buffalo semen at 4 - 5 ^{o}C compared to skim-milk diluent and

particularly egg-yolk glucose bicarbonate. Although the conception rate resulting from semen stored in any of the three mentioned diluents does not vary significantly because of the diluent factor, yet there is a noticeable tendency for better fertility from semen preserved in milk diluent. Besides, the average number of inseminations per conception is always lower (1.77) for semen diluted in milk than in egg yolk glucose bicarbonate (1.97) or egg yolk citrate (2.05). A diluent composed of 2.0 g lactose (20 ml of a 10% solution), 0.6 g sodium citrate dihydrate (24 ml of a 2.5% solution), 0.576 g anhydrous sodium phosphate (36 ml of a 1.6% solution) and 20 ml egg yolk per 100 ml of distilled water was developed. This is probably one of the very few extenders designed especially for buffalo semen. It is able to support sperm viability characteristics than do other commonly used diluents.

12.1.4.5. Freezing of buffalo semen

For 1183 ejaculates obtained from 8 bulls, the prefreezing and post-thawing motilities differed only slightly with sperm losses due to procedural treatments of about 16%. Some frozen ejaculates showed much better resistance in withstanding low temperature effects and displayed better survival rates at thawing; higher than 65% with sperm losses lower than 5%.

A wide array of extenders originally evolved for bull semen was, and still being, used in "hit-and- miss" empirical fashion. There is no diluent which has been composed specially for buffalo semen freezing that has taken into consideration the peculiarities of semen of this animal species. Because of this and of other associated factors like cryoprotectant level, equilibration period, freezing rate, thawing regime and differences in freezability of semen from individual bulls, reported cryopreservation results have always shown great variation and have often been contradictory. As with chilled semen, milk has been found better than egg yolk citrate even after 8 months of storage in liquid nitrogen. In a comparison between Tris -citric acid -fructose-yolk (TCFY), egg yolk citrate (EYC), egg yolk- glucose- bicarbonate (EYGB), egg yolk- skim milk (EYSM) and Laiciphos-271, EYSM and Laiciphos yielded higher post-thaw motility than other diluents. Sperm recovery in skim milk diluents has even been better than in Triladyl- a commercial Tris-based extender. Post-thawing motility in Tris buffer has been better at pH 7.0- 7.5 than at 6.5 or 8.0, but Tris molarities between 0.20 and 0.35 did not vary.

As with bull semen, glycerol concentration in the semen diluent has been shown to play a decisive role in post-thawing sperm recovery and viability. Concentrations of 6.4% in Tris, or 7.0% in skim milk appear better than lower or apparently higher concentrations. For pellet freezing of buffalo semen in lactose diluents, 2 - 3% glycerol results in better sperm revival. Although glycerolation at 4-5 ^{o}C is a common practice, satisfactory post-thawing motility and least morphological and biological damage have been recorded after room temperature glycerolation of buffalo semen processed in Tris.

Thawing of buffalo semen at relatively fast rates (50 ^{o}C for 15 sec or 35 ^{o}C for 30 sec) was found superior to thawing at slow rates (20 ^{o}C for 1 min or 5 ^{o}C for 2 min)**.**

No particular difference in post-thawing revival has been noted between freezing in ministraws or in minitubs as packages for frozen semen in spite of the differences between the two packages in the ratio of their surface to volume.

Freezing procedure results in significant reduction of sperm head length, maximum breadth, base width, acrosome length and head area. The bulk of evidence indicates that changes of sperm head measurements (except acrosome length) are primarily due to the combined effect of dilution, cooling, glycerolation and equilibration prior to freezing process.

Buffalo spermatozoa have been believed to be inherently more fragile than bull spermatozoa that upon freezing and thawing they are subjected to ultrastructural damage and subsequent detrimental chemical changes in the molecular organization of their membranes with leakage of vital material important for fertilization. Electron microscopic investigations indicate that ultrastructural changes start at the very initial steps of processing, mainly after the addition of glycerol and increase progressively with each further steps of processing. However, the sequential increase of ultrastructural damage after dilution, cooling, glycerolation and cold equilibration does not correlate in any way with the little change of sperm motility observed after these steps of processing. The first changes due to freeze processing are relevant mainly to the acrosomal region of the sperm that shows distention and loosening of the peri-acrosomal plasmalemma followed at late steps by ruffling and swelling. Plasmalemma overlying the post-acrosomal sheath is rather resistant. Drastic alteration of sperm ultrastructure, particularly of sperm nuclei and mitochondrial sheath, become evident mainly after freezing and thawing.

Visual estimation of sperm motility is in most cases used for appraisal of frozen semen quality. A number of reports recorded significant positive correlations between post-thawing motility on one hand and each of viability index, cervical mucus penetrability, and percent live sperm that passed through a column of sephadex and percent sperm displaying gelatinolytic activity. Negative correlations were also found between motility and the percent morphologically damaged acrosomes, bent tails and amount of GOT released from the spermatozoa in the extracellular media. In spite of being statistically significant, the obtained correlation coefficients were not high enough to be useful in predicting semen quality from motility estimates.

Fertility results from frozen buffalo semen vary significantly due to a number of factors. The same factors that control the fertility results of frozen bull semen seem to be involved also here. The individual variation in freezability of semen appears to be of prime importance. Differences of 42.33 - 70.68% among bulls was reported indicating that the choice of buffalo bulls whose semen can withstand the hazards of freezing and thawing can increase the net fertility rate from frozen semen.

12.1.5. Artificial insemination in sheep and goats

12.1.5.1. Semen collection

The ram will approach the restrained ewe and the collector should be positioned near the ewe, ready to collect with a properly prepared AV. The ram will normally smell, lick, and paw at the ewe. When the ram mounts, the collector should deflect the prepuce of the ram to the side, allowing the

penis to deviate into the AV. The penis itself should not be handled. Problems that may occur with collection with the AV include failure to ejaculate due to improper AV temperature or pressure or improper collector technique; delayed ejaculation after the AV is removed, usually due to poor technique or to over- or under-stimulation; and lastly, premature ejaculation due to poor technique, high libido, or overteasing. Rams can be trained to mount a teaser ewe and ejaculate into an artificial vagina with a collector kneeling beside him. Ejaculates may be collected daily from healthy mature rams, but sperm concentration and quality should be evaluated on a daily basis and collections adjusted accordingly to achieve optimal use of the ram.

Electroejaculation of the ram is a relatively simple and easy procedure with the correct equipment. In rams that are going to be used extensively in an AI program, it cannot be recommended because of the repeated stresses on the animal, but for minimal collections or animals that are too debilitated to mount a teaser, it is a viable alternative for the practitioner. It is also useful when examining a large number of rams in a day, for collection of vasectomized rams before use or for rams that refuse to serve an AV. Rams may be collected with or without sedation depending on their disposition, skill of the operator and assistants, as well as the frequency and objectives of the collection. Xylazine (15–20mg IM) or acepromazine (10mg IM) have been very effective. The ram should be restrained in lateral recumbence and the penis exteriorized (may be easiest to accomplish while the ram is set up on its dock and then lowered to lateral recumbence). Individual rams vary in their response, but generally, ejaculation occurs after 3 to 5 stimulations and if collecting for freezing and maximum sperm numbers are desired, stimulation may continue for several more stimulation sequences until ejaculation is not apparent or sufficient semen is obtained. If no ejaculation occurs after several stimulations, the probe should be removed and cleaned of feces and the battery checked on the electroejaculator.

12.1.5.2. Artificial insemination

12.1.5.2.1. Handling, assessment, and evaluation

Semen must be handled carefully to avoid heat shock; cold shock; contamination with water, disinfectants, sunlight, and air; and any other process or product that may decrease viability. Sperm will die if temperatures exceed 45°C and any increase above 37° C will increase metabolic rate and thus decrease sperm life. Cold shock may be avoided if all equipments that semen comes into contact with are kept at 30 to 37° C. All containers that come into contact with the semen should be either plastic or glass. Semen should be examined as soon as possible after collection and transferred to a sterile collection tube held in a 30° C water bath. Color should be milky off-white. Pink usually indicates contamination with blood, and a gray or brown color may be indicative of a reproductive organs infection. Urine contamination is usually yellow with a characteristic odor and the semen will appear dilute. Volume will range from 0.5 to 1.5 ml and may vary based on frequency and type of collection. Repeated collections over several days will decrease volume. Motility will normally be wave-like on gross examination under low power. An average ejaculate will contain 3.5 to 6.0 billion sperm per milliliter. Other tests that may be useful include a morphologic and acrosome integrity test using eosin-nigrosin stain or hypo-osmotic swelling tests. Individual sperm motility and progressive

forward movement by dilution of the sample with extender, sodium citrate, or phosphate buffered saline.

2.1.5.2.3. Dilution and insemination dose using fresh semen

A mature fertile ram normally deposits approximately 3 billion sperm. Doses of semen containing 100 million sperm have achieved acceptable conception rates when deposited into the cervix. If undiluted, this small volume is very hard to handle and measure accurately. Dilution not only solves this problem, it provides the sperm with a suitable environment for preservation during the insemination and handling process. Various extenders are available for fresh dilution, but the two most commonly used are heat-treated cow milk or Dulbecco's phosphate buffered saline. The extender must be either cooled or warmed to 30°C and then added slowly to the extender as soon as possible after collection and used as soon as practical (within 1 hour).

12.1.5.2.4. Freezing semen

There are many protocols for freezing ram semen. One step and two-step methods have been described, but because of its ease, the one-step method is the most commonly used. Semen is diluted to the final prefreezing dilution rate at 30° C with the cryoprotectant. Straws are then loaded and cooled slowly (using a water bath) by placing them in a refrigerator and cooling to 5°C over 1.5 to 2 hours. One formula that can be used is composed of tris (hydroxymethyl) aminomethane (24.2g) citric acid (13.6 g) fructose (10.0 g) glycerol (64 ml) egg yolk (200ml) in sufficient quantity to produce 1 L at a pH of 6.8. Commercial extenders have been used with success by the authors and for small numbers and ease of use are very satisfactory. Freezing semen in straws is similar to freezing bull semen, but the extender, dilution rates, cooling rates, and other factors will vary from laboratory to laboratory. Ram semen is more difficult to freeze than bull semen and some rams (5–10%) will not freeze successfully with current techniques and extenders that are available. Normally ram semen is diluted to at least 1:8 with the chosen extender with final dilution depending on the type of insemination (laparoscopic versus cervical versus transcervical AI). Semen can be loaded into straws or cooled before loading into straws, then cooled slowly to 4^{o}Ç to 5°C over a period of 1.5 to 2 hours. Depending on the laboratory or extender, semen is then either frozen over nitrogen vapor (4 cm for 0.25-ml straws or 6 cm for 0.5-ml straws) on a chilled (5° C) rack or held for several more hours to allow equilibration and then frozen as described previously. After 8 to 10 minutes in the vapor, the semen is then plunged into the liquid nitrogen and from there loaded into canes and transferred to a tank for storage using standard techniques. When using a programmable freezer, chilled filled straws are loaded into the freezer and cooled at the following rate: 4°C per minute until semen reaches −12°C, then 40°C per minute from −12° to −40°C, then finally 50°C per minute from −40° to −140°C. After this point is reached, straws are loaded into canes and stored as described previously. Semen can also be frozen in pellets by using a block of dry ice into which small depressions have been punched using a metal rod or die for multiple depressions. Semen frozen in pellets is usually diluted to only 1:3 to 1: 4, depending on sperm evaluation. Then 0.1 to 0.15ml of semen is pipetted into each depression and after 2 to 3 minutes the pellets are transferred to plastic goblets, labeled, and put in liquid nitrogen. For thawing, pellets are put into an extender prewarmed to 40°C, or two to three pellets are placed in a sterile dry test tube or Whirl Pak bag and put in a water bath at 40°C and shaken or stirred vigorously

till the pellets melt. The semen is then transferred to a 30°C water bath, where it may be held for up to 18 hours, till insemination. This technique does not require expensive or sophisticated equipment, but labeling and identity of the semen are harder to maintain. Frozen ram semen can be expected to achieve conception rates of 65% when using laparoscopic intrauterine AI.

12.1.5.2.5. Methods of insemination

Using fresh semen, vaginal and cervical deposition may result in acceptable pregnancy rates if using the appropriate dose of semen. Using frozen semen, intrauterine insemination is currently the only way to achieve acceptable pregnancy rates. Sheep have a complex cervical canal that is approximately 7 cm long with a series of 6 to 8 rear-ward facing, offset rings that make transcervical insemination difficult, if not impossible, because the cervix cannot be digitally manipulated per rectum as in the cow.

Vaginal insemination using fresh or chilled semen is done after cleaning the vulva (do not use disinfectants or other spermicidal agents) and inserting the insemination pipette along the dorsal wall of the vagina to the anterior vagina and depositing the semen there. This is also referred to as the “shot in the dark,” or SID, method. Pregnancy rates with frozen semen are not acceptable, but the technique is very fast; and fresh or chilled semen may yield acceptable rates, but it is a very inefficient use of semen.

Cervical insemination is performed with the ewe restrained “over the rail” by an assistant and the use of a speculum, head light, and angled tip insemination gun. The speculum is inserted to approximately 12cm with the jaws parallel to the lips of the vulva, and then rotated 90 degrees with its jaws opened. The cervical os is located and the tip of the insemination pipette inserted as far into the cervix as possible without using excessive force. The speculum is then withdrawn or closed, semen is deposited, and the insemination pipette is withdrawn so as to exclude any air and help keep semen backflow to a minimum.

Transcervical AI requires special positioning of the animal, cervical retraction and stabilization, and the use of specially designed instruments for the stabilization and passage of the insemination pipette through the cervix. Ewes are restrained in dorsal recumbence in a fetal-like position to ease stabilization and retraction of the cervix. A foot trimming table may work well for this as well as a specially designed crate. A vaginal speculum (30 mm outside diameter) with light source is lubricated and introduced into the vagina. The speculum should have a 1-cm opening along one side for instruments to pass. The cervix is identified and a pair of Bozeman forceps is introduced to grasp the tissue near the cervical os. This part of the procedure is critical and requires much expertise and training for consistent success. Correct attachment of the forceps facilitates entry and passage of the insemination equipment through the cervix. The cervix is then retracted and the handle of the forceps is passed through the side opening in the speculum so that the cervix can be better visualized. The bent-tipped preloaded insemination gun (modified Cassou) is then introduced into the cervical canal and rotated continually while attempting to negotiate through the cervical rings. Manipulation of the cervix at the same time with the forceps may improve or help passage through the rings. Once the cervical rings are penetrated, semen is deposited in the uterus, the pipette withdrawn, cervix released,

and the speculum removed. Trained, experienced inseminators may penetrate the cervix in as much as 75% to 85% of ewes, more in accelerated lambing programs. Cervical injury, abscesses, infections, and poor pregnancy rates are all associated with this technique, but vary by operator, semen dose, ewe condition, and experience. Pregnancy rates are generally lower than with laparoscopic AI, but this procedure may be a viable alternative when reduced pregnancy rates are acceptable.

Laparoscopic intrauterine insemination is performed using specialized equipment and small amounts of semen. It has a success rate of approximately 65%. The main disadvantages are the need for expensive laparoscopic equipment, invasive surgery, and the technical expertise needed to perform the procedure. Ewes should be held off feed for 24 to 36 hours and off water for 12 to 18 hours to decrease rumen fill, decrease chances of regurgitation, and reduce rumen size to allow easier visualization of the uterus. Equipment, personnel, pens, and handling devices should be arranged so that the procedures are done as quickly and efficiently as possible. Ewes are typically sedated 30 to 45 minutes before insemination with 5 to 10mg of acepromazine IM and stressed as little as possible. Ewes are restrained in a laparoscopic cradle in dorsal recumbence and securely restrained. Wool 10 to 20cm cranial to the mammary glands is surgically clipped and the skin is prepared for surgery using standard surgical technique. A local anesthetic is injected at the two penetration sites approximately 5 to 7cm cranial to the udder and 3 to 4cm on each side of the midline. Care should be taken to avoid major blood vessels. At this point, the cradle is tipped up so that the ewe is on a plane at approximately 45 degrees with the head down. The abdominal organs are displaced cranially to allow better visualization of the bladder, rectum, and uterus. The gas trocar is introduced into the abdomen through one of the anesthetized areas, and CO2 is used to insufflate the abdomen to the satisfaction of the operator, and then the other trocar is introduced through the other anesthetized area. Both trocars are removed and the laparoscope is inserted into one of the ports and the internal organs are identified. The uterus is usually located just under the bladder or under the caudal omental fat. After the uterus is identified and moved into position if necessary, the loaded insemination pipette is introduced and directed toward the uterus midway between the bifurcation and the uterotubal junction. A quick stab is made into the lumen and one half the dose is deposited in one horn and then the procedure repeated in the other horn. After insemination, the insemination pipette is removed, then the laparoscope. The abdomen is deflated and the cannulae removed. Bleeding can be stopped from the puncture sites with pressure, suture, or staples. Some operators close all punctures with suture, and others only close those that need it. Prophylactic antibiotics may be given as well as protection for tetanus. Instruments should be sanitized between animals by bathing in a suitable antiseptic such as 0.5% benzalkonium chloride. An experienced team in a well-equipped and organized operation may be able to do 300 or more ewes in a day using this technique.

12.2. Embryo Transfer

In farm animals, fertilized ova is removed from the uterus of their dam (the donor) and transferred to the uterus of other females (recipients) for development to term. The main use of embryo transfer is increased productivity of selected females; others are identification of potential artificial insemination bulls through contract matings, disease control, importation and exportation of livestock, rapid

screening of AI sires for genetically recessive characteristics, and treatment or circumvention of certain types of infertility. Embryo transfer also is a useful research tool for evaluating fetal and maternal interactions.

12.2.1. Embryo transfer in camels

12.2.1.1. Superovulation

Superovulation treatments, to stimulate the growth of multiple follicles, include the use of exogenous gonadotrophins such as equine Chorionic Gonadotrophin (eCG) or Follicle Stimulating Hormone (FSH) which may or may not be given after a period of progesterone priming. This progesterone priming can be given either as a progesterone releasing intravaginal device (PRID) inserted into the vagina for a period of seven days, or as daily injections of 100 - 150 mg progesterone in-oil for up to 15 days. However, the best results occur if the camel is treated with exogenous gonadotrophins when there is minimum follicular activity in the ovaries. If follicles are present at the time of treatment these tend to develop into overlarge follicles before the new stimulated wave of follicles have had a chance to develop.

i. Follicle stimulating hormone (FSH): in the dromedary a total dose of 18 mg of ovine FSH (oFSH) or 400 mg porcine FSH (pFSH) in 20 ml is given over 4 days. Generally speaking, two injections are given daily in gradually decreasing doses for example: Day 1: 2 x 4 ml, Day 2: 2 x 3 ml, Day 3: 2 x 2 ml, Day 4: 2 x 1 ml.

ii. Equine Chorionic Gonadotrophin (eCG): the dosage of eCG used varies from 1500 - 6000 iu. It is generally injected in a single dose one day before, or on the day of, completion of a 5 - 15 day progesterone regime.

iii. Combined eCG and FSH - the best response is seen when a combination of both FSH and eCG are given. The eCG (2500 iu) is given as a single injection on day 1 of treatment together with the first of the twice daily injections of FSH, followed by three more days of twice daily injections in decreasing doses of FSH as described above.

12.2.1.2. Problems with superovulation in camels

Superovulation treatments in the female camels are far from perfect as the ovulation response and embryo yield remain highly variable. The main problems are:

i. The high incidence of non-responsive females: approximately 20 - 30% of stimulated females do not develop follicles.

ii. The high incidence of follicle luteinization before breeding: this is particularly prevalent in eCG-treated females and could be due to the LH activity of this hormone.

iii. The high incidence of over stimulated ovaries: in some eCG or FSH stimulated females, the ovaries become very large and contain many generations of follicles of different sizes. This may be due to an individual difference in response to the hormones.

iv. Dromedary camels can become refractory to superovulation with FSH and eCG - this is probably caused by immunization against these hormones. We have observed a complete arrest of ovarian activity in some females that have been superovulated with these hormones repeatedly over several years.

12.2.1.3. Mating and induction of ovulation

In order to achieve a good ovulation rate, donors should be monitored by ultrasonography and palpation throughout the superovulation treatment and bred when the follicles reach a suitable size of between 1.3 - 1.8 cm in diameter. Follicles generally start to develop about 4 - 6 days after the start of treatment and reach 13 - 16 mm in diameter approximately 8 - 12 days after the start of treatment. The number of matings per donor can vary, may be twice at a 24 hours interval, and although ovulation occurs in response to mating, the donors are given a single intravenous (i.v.) injection of GnRH analogue (20μg buserelin) at the time of the first mating in order to maximize ovulation response.

12.2.1.4. Embryo collection and evaluation

The donor can either be placed in stocks or restrained sitting on the ground after being sedated. Then the rectum needs to be cleared of all faeces and the tail wrapped in a tail bandage before cleaning the perineal region thoroughly. Some people like to use epidural anaesthesia which can be advantageous in young dromedaries because of the smallness of the pelvis, however, in larger females it is not usually necessary, especially if they are already sedated.
The uterus is flushed using a Gibbon Balloon (20 Gauge) or Foley catheter (18 - 20 Gauge). Using a sterile gloved hand the catheter is guided through the vagina; then the cervix is dilated manually and the catheter inserted. Once the catheter is through the cervix the cuff is inflated with 30 - 40 ml of air or PBS medium and pulled back against the internal os of the cervix to seal it. The uterus is then flushed repeatedly with 60 - 120 ml of flushing medium, which may be either commercially available bovine embryo flushing media or Dulbecco's phosphate buffered saline (DPBS) + 0.2% Bovine Serum Albumin (BSA), + 0.005% (w:v) kanamycin sulphate. The uterus is palpated whilst being flushed to monitor uterine filling and when it feels fully distended the medium is collected, by gravity flow, into sterile beakers. The recovery of the medium has to be as complete as possible and this is aided by gentle massage of the uterine horns whilst collecting the fluid. The process is repeated at least 3 times or until a total volume of approximately 500 ml has been used. Some authors prefer to flush each horn separately because the cervical canal can relax during flushing and the cuff may slip back into the vagina causing loss of some flushing media. For flushing individual horns the catheter should be placed in the uterine horn so that the cuff is positioned in its lower third. This can be difficult to judge as the uterine horns in camelidae are separated internally by a septum that is not palpable. The cuff is inflated with air or flushing medium so that the catheter is well anchored and cannot move under the pressure of the medium. The horn is then flushed 4 to 5 times by injection of 30 - 120 ml of flushing media. The operation is then repeated for the other horn. The collected medium is filtered through an embryo filter until only 20 - 30 ml of medium remains. This is poured into a sterile petri dish and examined under a microscope for the presence of embryos. As many as 20 or more embryos have been recovered in a single flush but because not all the follicles will ovulate at the same time these embryos can vary greatly in size and development.

12.2.1.5. Effect of timing on embryo recovery rate

It is now well established that in camels the embryo does not reach the uterus until day 6 or 6.5 after ovulation (Day 0 = one day after mating). Therefore any attempt to collect embryos before day 6 post ovulation, results in low recovery rates. In practice the best recovery rates from dromedaries are achieved when the uterus is flushed on day 7 or 8 after ovulation.

12.2.1.6. Evaluation of embryos

Embryos recovered from the uterus in camelidae are generally at the hatched blastocyst stage. The evaluation system used by most authors classifies the embryos into 5 grades according to their morphological characteristics and stage of development. The clinician should look for abnormalities such as:

i. extruded blastomeres (i.e. individual cells which have been extruded from the cell mass);

ii. signs of degeneration (dark areas), and

iii. obvious morphological anomalies such as folding or wrinkling.

12.2.1.7. Management of recipients

The two main aspects of selection of recipients for embryo transfer are: (i) the screening of reproductive and health problems, and (ii) the preparation and synchronization with the donor.

Synchronization of the cycle in female camels has met with many difficulties due to the peculiar nature of follicular activity in these species. The best recipients should ovulate 24 - 48 hours after the donors.

Synchronization of ovulation between the donor and recipient can be approached using one of the following methods:

a. Selection of recipients from a random group. If using this technique a group of recipients at known stages of their reproductive cycles are examined 24 hours after the donor is bred and all females that have a mature follicle (1.3 - 1.8 cm in diameter) are treated with GnRH or hCG. This method of selection is time consuming and can only be used if the number of donors is limited.

b. Using progestagen treatments such as PRID or subcutaneous implants (Norgestomet). However, these methods have only limited success as they do not seem to completely arrest follicular development and therefore, have very limited efficacy in synchronizing follicular development.

c. Better results can be obtained when recipients are induced to ovulate with hCG or GnRH following a treatment combining progesterone and eCG. The recipients are treated daily with progesterone-in-oil (100 mg/day) for 10 to 15 days, to try and dampen the development of more follicles, and on the last day of progesterone treatment, 1500 - 2500 i.u. eCG is injected

to induce follicular development. Progesterone treatment is scheduled to end on the day of injection of gonadotrophin in the donor in an attempt to synchronize the recipient and donor. The eCG treatment guarantees the presence of mature follicles in the recipient at the same time or 24 - 48 hours after the donor.

d. Progesterone (100 mg) is given daily starting 2 days after mating of the donor. However, because there is no corpus luteum (CL), progesterone treatment has to be continued throughout pregnancy.

e. Bilaterally ovariectomized females can also be used as recipients. Females are treated for two days with estradiol 17-b (40 mg/day) followed by daily injections of progesterone (100 mg/day). This has resulted in 30% pregnancy rates but again has the disadvantage of daily progesterone injections having to be continued throughout pregnancy.

All recipients should be screened on the day of transfer to ascertain that ovulation has occurred and that a mature CL is present.

12.2.1.8. Transfer of embryos

Embryos can be transferred surgically or non-surgically. Surgical embryo transfer - surgical embryo transfer in the dromedary and Bactrian camels is done via the left flank laparotomy. The embryo is transferred into the uterine cavity through a puncture made in the exteriorized horn by a Pasteur pipette. However, this technique cannot be used in young and primiparous animals because the uterine horn is too short and is difficult to exteriorize. Non-surgical technique - the non-surgical technique for embryo transfer consists of placing the embryo directly into the uterine lumen through the cervix using a regular bovine insemination gun. The embryo is loaded into a 0.25 ml or 0.5 ml sterile plastic straw and placed in the gun for transfer. The inseminating gun is first covered by a sterile sheath with a side opening, so that the embryos can escape even if the pipette is up against the wall of the uterus, then this is covered with a second plastic sanitary sheath.

The recipient is prepared in the same manner described for embryo collection. Then the embryo is transferred as follows:

i. The inseminating gun is introduced into the vagina and guided towards the cervix using a sterile gloved hand.

ii. The sanitary sheath is perforated after passage of the first cervical ring, by pulling the plastic sheath backwards towards the technician, and the gun is further guided into one of the uterine horns with a hand in the rectum.

iii. The plunger of the transfer pipette is pushed home and the embryo deposited into the uterus.

The passage through the cervix and uterine deposition of the embryo should be done as quickly as possible to avoid excessive irritation of the cervix and uterine mucosa which may cause prostaglandin F2α release and CL demise.

12.2.2. Embryo transfer in horses

Whilst embryo transfer (ET) has become a widely-used and accepted technology in many farm species, there are major issues for the equine industry:

• The long follicular phase of the estrous cycle means it is difficult to synchronize ovulations.

• Difficulties in synchronizing mares may mean that a large group of potential recipients is required, making the process not inexpensive.

• Problems achieving superovulation in mares means that multiple embryos are rarely collected.

• There is legislation against insemination by certain registration authorities.

• Certain registration authorities will not register offspring resulting from ET. These issues are worsened by the current low success rate using stored embryos, although short-term storage is possible with some careful laboratory techniques.

12.2.2.1. Management of donor mares

• Timing of ovulation should be carefully recorded.

• Embryos are best recovered on days 7 or 8 after ovulation. The procedure involves the infusion and subsequent collection of a physiologically balanced solution into the uterus of the conscious mare.

(1) The mare's tail is bandaged and fixed to one side and the perineum should be aseptically prepared.

(2) A Foley-style collection catheter with a suitably-sized cuff (at least 50 ml) should be placed into the uterus with the cuff adjacent to the proximal cervix – this should provide an effective seal.

(3) 1–2 liter of medium containing fetal calf serum and antibiotic is instilled into the uterus, the uterus is gently massaged per rectum and the fluid is drained out into a sterile collection device, or through an embryo filter.

(4) The procedure is repeated 3–4 times.

12.2.2.2. Recovery and examination of the embryo

• The embryo is quite easily identified by examination at 20x magnification.

• The embryo is 'washed' by transferring through successive drops of new culture medium and finally placed in holding medium for careful examination.

• Poor-quality embryos have little chance of successful implantation.

• The embryo can be stored for up to 24 hours in special medium when cooled to 5°C.

12.2.2.3. Management of recipient mares

• Young fertile mares, with a normal breeding soundness examination, are required.

• Synchronization is best planned to ensure that the recipient ovulates 1–3 days after the donor (i.e. a 7-day embryo is implanted into a mare 4–6 days after ovulation).

• Common techniques are to have a cohort of three or four potential recipients that are synchronized to the donor using prostaglandin administration.

• Careful monitoring of the recipients is required to determine the timing of ovulation in each.

• Selection of the recipient is based on which ovulates at the correct time and has the most normal cycle.

NB: Poor success rates can be expected if the recipient has ovulated before the donor, and anecdotally in some cases when estrus synchronization has been attempted using progestagens.

12.2.2.4. Transfer to the recipient mare

• Non-surgical transfer involves catheterization of the cervix using a guarded pipette. It is important to ensure that the embryo is not left within the pipette and usually it is placed within a small volume of medium with strategically-placed air gaps between this and other drops of medium.

• Surgical transfer is normally performed in the sedated mare via a flank laparotomy.

12.2.2.5. Post-transfer management of the recipient

Some clinicians administer progestagens to the recipient mare in an attempt to encourage embryo survival and delay possible luteolysis due to delayed maternal recognition of pregnancy.

12.2.2.6. Preservation of embryos

• Generally there have been low success rates for embryos that have been cryopreserved.

• The cryopreservative, glycerol, seems to have a significant toxic effect.

• Up to 40% success rates have been demonstrated after frozen-thawed embryos have been transferred (but low numbers of animals have been used in these studies).

• Short-term preservation (up to 24 hours) is more successful.

12.2.3. Embryo transfer in cattle

The reproductive potential of each normal newborn calf is enormous. There are an estimated 150,000 potential “eggs” or ova in the female and countless billions of sperm produced by each male. By natural breeding, only a fraction of the reproductive potential of an outstanding individual could be realized. The average herd bull will sire 15 to 50 calves per year and the average cow will have one

calf per year. With artificial insemination, it is possible to exploit the vast numbers of sperm produced by a genetically superior bull; however the reproductive potential of the female has been largely unutilized. She will produce an average of eight to 10 calves in her entire lifetime under normal management programs. Like artificial insemination has done for the bull, embryo transfer is a technique that can greatly increase the number of offspring that a genetically important cow can produce. The main reason for the development of embryo transfer in cattle was to further the increase in genetic progress made possible by the adoption of AI.

12.2.3.1. Embryo transfer procedures

12.2.3.1.1. Preparation of donor cows

Superovulation is induced by administering exogenous follicle stimulating hormone (FSH) However, since the half-life of FSH in the circulation is relatively short, repeated injections are required, usually over a five-day period. However, most superovulation in preparation for embryo transfer is induced using pregnant mare's serum gonadotrophin (PMSG) which has been found to have both FSH-like and LH-like properties, but has a much longer half life in the body (in excess of 50 hours as compared to approximately 0.5–1 hour for endogenous LH and FSH). The response of individual cows to PMSG is highly variable, but is to some extent dose-dependent. The number of ovulations produced normally ranges from one to well over 20. When large numbers of ovulations are induced the recovery of ova is less efficient, perhaps because their passage into the fimbria after ovulation is impaired. The proportion of normal fertilized eggs is also reduced. The usual dose is between 1500 and 3000 international units (IU) given as an intramuscular injection in a small volume of saline. It is known that the presence of a dominant follicle can disrupt superovulatory response in cattle. Destruction of the dominant follicle by trans-vaginal aspiration of its contents (as used in ovum pick-up) enhances ovulation rates and yields of viable embryos.

A typical regimen as used in preparing donors is as follows:

- PMSG is given between days 9 and 12 of the cow's natural cycle.
- Ovulation is induced at a predetermined time by the injection of prostaglandin or an analogue to cause luteolysis. The cow is expected to come into heat within a 24- to 96-hour period.
- Artificial insemination is carried out on the day of estrus using one 2.5-ml straw of semen. On the same day a second insemination is carried out using two straws of semen. Most authors report mean ovulation rates of between 8 and 18 ovulations and a fertilization rate of recovered eggs of around 80%.

12.2.3.1.2. Collection of embryos

Until the mid-1970s most successful embryo recoveries were carried out by means of surgery under general anaesthetic. Non-surgical collection via the cervix generally results in the recovery of around 10% fewer embryos, but is cheaper and simpler, avoids the risks of general anaesthesia and can be carried out on farms. Thus, most commercial embryo recoveries are now carried out non-surgically.

Recovery via the cervix is only possible after the embryos have entered the uterus, and is thus normally carried out at or soon after day 6 of the oestrous cycle following superovulation. The donor cow is restrained in a normal cattle crush, usually with her front feet higher than her hind feet to render the tract more accessible. The cow is usually tranquillized and given an epidural anaesthetic injection, after which embryos are flushed from the uterus using a Foley three-way catheter. The catheter has three channels: one for the admission of air to inflate a collar near the tip, one with an outlet very close to the tip for the entry of flushing medium and one with an opening slightly further from the tip for the collection of flushing media, plus any recovered embryos, from the uterus. The sterile catheter is inserted into the first uterine horn to be flushed (usually ipsilateral to the ovary which appears to have shed most ova), being protected from contamination in the vulva and vagina by means of sleeves which do not pass through the cervix. Once the catheter is in position, the collar is inflated by means of a syringe filled with air; this serves to hold the catheter in position and to prevent the leakage of flushing medium into the body of the uterus. A large volume of flushing medium (200–500 ml depending on the size of the cow or heifer) is injected by means of a syringe into the first channel and allowed to flow back through the outlet channel into a large test tube or glass cylinder. The catheter is then repositioned in the other uterine horn so that it may be flushed in the same way. The whole operation is facilitated by manipulation of the reproductive organs per rectum. Commercially available Dulbecco's PBS (phosphate buffered saline) ova culture medium, to which is added 4 mg of bovine albumin, is used for flushing the tract and also for subsequent short-term storage and transfer of embryos. Embryos are slightly denser than the culture medium and thus tend to sink to the bottom of the collecting vessel, usually within two minutes. In practice, the collection vessels are allowed to stand for 10 minutes, after which it has been found that 98% of the embryos in the medium will be recovered. Most of the medium can be siphoned off after the embryos have settled so that they may be expected to be found in the remaining 10–20 ml. The embryos are just visible as small specks to the naked eye and can be seen and manipulated in small quantities of medium in glass Petri dishes under a low-power microscope.

12.2.3.1.3. Storage of embryos

If embryos are to be transferred to recipient cows or heifers on the same day they may be stored in a fresh, sterile solution of the same culture medium for several hours, provided they are maintained close to body temperature. It is now possible to freeze cattle embryos for long-term storage, after which pregnancy rates in excess of 50% can be expected under the best conditions. The problems involved with freezing embryos are analogous to those of freezing semen, except that embryos are more sensitive to the freezing and thawing process and high wastage rates cannot be tolerated. As with spermatozoa, glycerol is often used as a cryoprotective agent. Embryos are normally stored individually in 0.25-ml straws such as are used for AI and are then reduced to liquid nitrogen temperature at a controlled rate. After storage the embryos must be returned to body temperature at a controlled rate related to the speed at which they were originally cooled. In practice the straws are thawed in 37°C water baths. Cryoprotectant then needs to be removed. This is carried out in stages to avoid osmotic shock. More recently, simplified methods of cryopreservation have been developed. These include a one-step method, direct transfer after thawing, vitrification and ultra-rapid freezing.

12.2.3.1.4. Preparation of recipients

For the immediate transfer of fresh embryos, donor and recipient should have been in estrus on the same day, or within a day of each other. This can be achieved by synchronizing the recipient with an injection of prostaglandin or an analogue. If enough potential recipients are available it may be possible to select cows that have been observed in natural estrus at the appropriate time.

12.2.3.1.5. Transfer of embryos

Individual embryos are usually drawn into the straws by means of a small syringe. Non-surgical transfer of embryos is carried out through the cervix, using a longer version of the apparatus used for AI. A very similar technique is employed, except that greater care is taken to avoid contamination of the inseminating gun in the vagina. The embryo is also deposited further into the uterus – as far as possible into the horn ipsilateral to the ovary that ovulated.

An average of five embryos per flush can be expected using non-surgical recovery methods, leading to an average of 50 freezable embryos per donor per year and the birth of around 30 calves. About half of these could be of lesser value because they are of the wrong sex.

12.2.4. Embryo transfer in buffaloes

There are two major criteria for the selection of donor animals, genetic merit and reproductive performance. The donor buffalo must be in good body condition and preferably gaining weight. It should be free from underlying diseases, a minimum of 50–60 days post-partum and cycling regularly. Generally, buffaloes with a history of reproductive problems will not make good donor animals. Donors are further evaluated by rectal examination of the cervix, uterus and ovaries to determine that they are free from adhesions or other palpable lesions. It is prudent to test the patency of the cervical canal with a cervical dilator for a sufficient diameter to permit passage of a collection catheter. This prevents the occasional frustration of being unable to negotiate the cervix after a series of costly hormonal injections, particularly in nulliparous animals.

12.2.4.1. Donor treatment

For optimal efficiency two to four donors should be treated and synchronized with their recipients for each attempt; this allows the sharing of the recommended potential of three to four recipients per donor. Follicle-stimulating hormone (FSH) or pregnant mare serum gonadotrophin (PMSG) may be used. Treatment is begun during the mid-luteal phase (day 8 to 12) of the donor's cycle and employs the use of prostaglandins ($PGF_2\alpha$) to synchronize the cycles of the donors and the recipients. Dose levels of FSH vary from decreasing levels of 6, 5, 4, 3 and 2 mg twice daily to a uniform 4 or 5 mg twice-daily injection schedule. $PGF_2\alpha$ (25–35 mg $PGF_2\alpha$ or 500 mcg $PGF_2\alpha$-analogue IM) are routinely given at the time of the fifth and sixth FSH injection which is then followed by estrus and ovulation in two and three days, respectively. This interval from $PGF_2\alpha$ to the onset of estrus is 12–24 hours shorter in superovulated animals than in naturally ovulating buffaloes. Consequently, recipients should be injected with $PGF_2\alpha$ 24 hours before the donors if this method of synchronization is used. The response to the foregoing FSH regimens ranges from zero to as many as

15 ovulations, with an average of five or six. There appears to be no difference in response between a four-day and a five-day regimen. Pregnant mare serum gonadotrophin (PMSG) has also been used for superovulation. While PMSG has the advantage of requiring only one single injection, its half-life is perhaps too long as measurable levels could still be detected in the blood of cattle 10–15 days after administration of 1500–3000 IU PMSG. When used in conjunction with prostaglandin, PMSG is administered between days 8 and 15 of an estrous cycle followed by prostaglandin 48–72 hours later. A promising new development in the superovulation of cattle with PMSG is the administration of anti-PMSG at the time the donor comes into estrus. This counteracts the continued stimulation of follicles due to the long half-life.

12.2.4.2. Estrus detection and insemination

If natural service is used, the donors should be exposed to the male starting just prior to the time of the last scheduled FSH injection (day-1, p.m.) and continued at eight-hour intervals for 10 minutes until they are no longer receptive. With artificial insemination, the donors should be inseminated twice with a 10–12 hour interval beginning 8–10 hours after the onset of estrus, to cover the range of time over which the ovulations may occur. Depending on the quality of the frozen semen, a double inseminating dose may be used at each insemination.

12.2.4.3. Flushing and holding media

Two types of media are used for the recovery of embryos, phosphate-or bicarbonate-buffered solutions. The pH of most body fluids is 7.2 to 7.4. A pH range of 6.9 to 7.7 is compatible with normal embryo development in mice. Osmolarity of the medium should be in the range of 270–300 mosm. The most commonly used medium for non-surgical embryo recovery is Dulbecco's phosphate buffered saline (PBS). One-percent heat-treated bovine serum (10 ml) is added to each individual 1-litre bottle of flushing medium which has been warmed to a temperature of 30–37°C. Serum may act as a protein source for embryo growth and membrane stabilization, and renders the embryos less sticky. Heat treatment at 56°C for 30 minutes removes complement which is embryotoxic. In lieu of serum, 0.4 percent bovine serum albumin may be used for recovery and holding media. The advantage of phosphate-buffered media is that they maintain the pH during exposure to the air. Bicarbonate-buffered media need a gas phase of 5 percent CO_2 which means that they require a closed system. Upon exposure to air, CO_2 rapidly escapes leading to a rise in pH. Examples of bicarbonate buffered media are Ham's F10, Brinster's BMOC-3 and TCM 199 (Hepe's). Glucose or dextrose and pyruvate are actually only necessary for longer periods of embryo culture. For flushing, PBS only needs to contain penicillin and 1–2 percent serum. Serum is added to the flushing medium in a rate of 10–20 percent to make a holding medium which can also be used for short-term (less than 24 hours) culture. Holding medium should be sterilized by filtration through a 22-μ or 45-μ millipore filter attached to a large glass syringe. The rubber plungers of some syringes have been shown to be coated with an embryotoxic lubricant.

Factors which may affect embryo viability during short-term culture include pH, osmolarity, (elevated) temperature, contamination and toxicity of the medium. Embryos should be stored in the same type of medium that was used for flushing. Changing embryos from a phosphate- to a

bicarbonate-buffered medium is undesirable because of the possible changes in osmolarity, pH and energy substrates. Holding dishes must be kept covered to minimize contamination and evaporation. The latter leads to an increase in the osmolarity of the medium. Changing the embryos to a fresh dish of holding medium from time to time further minimizes the effects of contamination and evaporation.

12.2.4.4. Non-surgical embryo recovery

Bubaline embryos are believed to descend into the uterus around day 4 (estrus = day 0) and shed their zona pellucida ("hatch") between days 6 and 7. Consequently, most non-surgical recoveries should be made on days 5 and 6. Buffalo embryos have been recovered non-surgically by Foley catheter.

A two-way roundtip Foley catheter (French size No. 16 to 24) with a 30-ml inflatable balloon is used. The two-way catheter has one channel for inflation of the balloon and a single channel for alternate inflow and outflow of flushing medium. A sterile stylet (such as the plunger of an insemination gun) is inserted the full length of the device to render it sufficiently rigid to allow introduction into the uterus under guidance per rectum.

The donor is restrained in a chute or in stocks. Nervous animals are given 5–10 mg of chlorpromazine or another suitable tranquilizer. Feces are carefully removed from the rectum to avoid aspiration of air, and a preliminary estimate is made of the number of ovulations (CL). Epidural anaesthesia is administered (4–6 ml of 2-percent lidocaine) to prevent defecation and straining. Inadvertent air can be removed from the rectum with a small stomach tube attached to a "wet vac" vacuum cleaner. The vulva and perineal region are thoroughly washed with plain water and blotted dry. The tail is tied out of the way. If the cervix feels small or tortuous, a cervical dilator may be used to gently expand and straighten the cervical canal. The dilator and subsequent catheters are covered with a sanitary sleeve before they are introduced into the vagina. This protective cover is perforated just before the instrument enters the external os of the cervix. The rigid, relatively sharp-pointed dilator should be used with extreme caution as it can readily perforate the uterine wall as it is "forced" through the tight cervical canal. The lips of the vulva are again parted and the covered Foley catheter, with the stylet in place, is inserted into the vagina and on into the lumen of the cervix. It is then manipulated into the appropriate horn until the inflatable balloon is situated at the base of the uterine horn. The balloon is slowly inflated with 15–25 ml of air in adult buffaloes and 10–15 ml of air in heifers. The endometrium can easily be split by overdistension, resulting in haemorrhage and escape of the flushing solution into the mesometrium from which it cannot be recovered.

After the catheter is in position, the stylet is removed and the catheter is connected via a Y-junction by sterile tubing to a 1 - 000 ml bottle of flushing solution. The remaining arm of the Y-junction is connected to a free piece of tubing. The flow of medium in both pieces of tubing is controlled by quick-release clamps. While the outlet tubing is occluded, the flushing solution enters the uterus by gravity flow with the bottle suspended one meter above the level of the uterus. The horn of the uterus is extended by elevating the utero-tubal junction and by carrying it anteriorly. When the inflow stops, the inlet tubing is clamped off and the clamp on the outlet tubing is released. The fluid is channelled directly through an embryo filter (75-μ pore size).

Alternatively, when filters are not available, the effluent may be collected in a 1-litre graduated cylinder. After the embryos have been allowed to settle for 20–30 minutes, the supernatant is carefully siphoned off and the last 75 ml are examined directly under a stereomicroscope. Slow siphoning is most easily accomplished by gently lowering a length of small diameter (e.g. 1 mm) tubing into the cylinder until the end of the tubing reaches the 75-ml mark. Siphoning is started by aspiration with a small syringe at the other end of the tubing.

In older animals with long pendulous tracts, manipulation of the cervix and uterus is facilitated by retracting the cervix into the vagina with cervical forceps. If the returning fluid is blood-tinged, the red cells may be washed directly through the filter by opening both clamps between the filter and the bottle of flushing solution. The filter should never be allowed to run completely dry leaving the embryos on the filter disk exposed to the air. A 1-cm layer of fluid can be maintained by regulation with a clamp on the tubing attached to the bottom of the filter unit. During the final collection of the flushing solution, 50 IU oxytocin may be given to aid in the recovery of the residual portion of the medium from the uterus.

12.2.4.5. Embryo handling and evaluation

Once removed from the stable, the embryo should be handled with respect regarding its temperature, pH, osmolarity and contaminant factors which may affect viability.

Embryos may be maintained at room temperature for several hours without decreasing pregnancy rates significantly, provided the embryos are transferred to fresh holding medium every two hours. Storing embryos in an ice-water bath is recommended for long-distance transportation. There is no obvious decrease in pregnancy rates after storage at this temperature for 12–24 hours. Embryos do not tolerate temperatures above body temperature (39°C) very well.

The embryo is spherical and is composed of cells (blastomeres) surrounded by a gelatin-like shell, an acellular matrix known as the zona pellucida.

The stage of development after fertilization must be appropriate for the day on which the embryo is collected, but is not precisely known for buffalo embryos since only relatively few have been recovered to date, with most of them recovered on day 5 to day 7 after the onset of oestrus. The rate of development of water buffalo embryos is faster than that of cattle embryos. Development from the morula stage to escape from the zona pellucida (hatching) appears to occur in one day in buffaloes. Morphologically water buffalo embryos are similar to bovine embryos and general morphological descriptions can be adapted.

12.2.4.6. Handling of embryos

Once an embryo is identified in the searching dish, it is immediately transferred to a small Petri dish (35×10 mm) containing fresh, filtered (0.22–0.45 μ pore size), sterile medium. As a holding medium, generally phosphate buffered saline (PBS) containing penicillin plus 10–20 percent heat inactivated serum is used. Embryos are tentatively classified simply as good or bad, and may be recorded on the cover of the holding dish. This allows for a quick estimate of the total number of

embryos found. Embryos are then serially rinsed through at least three different dishes containing fresh sterile medium using a new sterile pipette for each step. Finally, they are placed in a dish awaiting transfer or cryopreservation. Under certain circumstances, e.g. for export, embryos *must be* rinsed through ten different dishes containing sterile media. All dishes must be kept covered between searches to avoid contamination, and particularly evaporation, when placed in the incubator. Evaporation of the small volume of medium in a flat dish rapidly leads to hypertonic solutions.

12.2.4.7. Recipient selection and synchronization

Recipients should be large-framed, healthy, mature young buffaloes in good body condition. A minimum of two normal cycles should ideally have been recorded before use whether they will be synchronized with prostaglandins or selected from a pool of cycling animals.

The availability of synchronous recipients can be achieved in two ways: (1) a large pool of cycling females, which limits the number of donors and time when they can be collected: 5 percent of the herd will be in heat on any given day; (2) a relatively small number of recipients can be synchronized with prostaglandin F_2 alpha (PGF_2 alpha) or its analogues to exhibit heat the same day or just ahead of the donor. Animals with a palpable corpus luteum are injected with 25 mg PGF_2 alpha or 0.5 mg PG-analogue IM and may be expected to come into estrus in two to four days with a peak on the third day. Alternatively, all recipients may be injected with prostaglandins regardless of the presence or absence of a corpus luteum. A second injection is then given 11 days later, and again estrus will peak on the third day after the second injection. The average buffalo donor yields two to three transferable embryos. Therefore, four recipients per donor is a reasonable number to prepare. Recipients must be injected with prostaglandins one day earlier than the donor. Following gonadotrophin treatment, the donor comes into estrus 48 hours after the prostaglandins, while the recipients that did not receive any gonadotrophin treatment will come into estrus 72 hours after prostaglandin treatment.

Not all donors will respond to the superovulatory treatment. For optimal efficiency, two to four donors should be superovulated at the same time to permit the sharing of the prepared recipients and avoid the expensive failure encountered too frequently when only one donor is prepared at a time.

Non-surgical transfers are made through the cervix as for artificial insemination. It is important to minimize contamination of the uterus because it is more susceptible to infection during the luteal phase. Faeces are evacuated from the rectum and the side of the corpus luteum is determined. Epidural anaesthesia is induced to prevent defaecation and to minimize straining. The perineal region is thoroughly washed and the vulva is blotted dry.

In the laboratory the embryo is aspirated into a 0.25-ml French straw between two air pockets and two columns of culture medium. The straw is inserted into the AI gun and shortened level with the end of the gun. A sterilized sheath is fitted over the AI gun and fixed in place. A second, sterile, larger (sanitary) sheath which is closed at the distal end is fitted over the first to serve as a cannula to permit passage of the gun through the vagina without coming into contact with the vaginal flora. The tip is placed into the external os of the cervix and is then pushed through the sheath before it is guided as

gently as possible through the remainder of the cervical canal and into the uterine horn on the side of the corpus luteum. The embryo is then gently deposited approximately one-third of the way ups the uterine horn and the gun is withdrawn slowly. A negative correlation has been demonstrated between the time spent manipulating the cervix and the uterine horn and the pregnancy rate. Success is related to dexterity and practice. Pregnancy rates achieved by most operators improve with experience. The pregnancy rate in cows after non-surgical transfer through the cervix is generally 10–15 percent lower than that reported after surgical transfer. In the hands of some experienced embryo transfer practitioners, pregnancy rates following non-surgical transfer approximate those after surgical methods.

12.2.5. Embryo transfer in sheep and goats

12.2.5.1. Expectations of embryo transfer

Response to super-ovulation: approximately 25% of programmed donors will not respond to super-ovulation treatments. Some never respond, some may respond on a subsequent program. If the donor responds, she may produce from 1 to over 30 embryos, with average 8 to 12 depending on the breed, time of year, condition of animal. This is one of the biggest "X" factors in any MOET program. Donors must be cycling for MOET programs to be consistent; therefore, extra diligence must be taken when programming donors outside of the normal breeding season. *Number of transferable embryos*: not all recovered embryos are capable of producing a pregnancy. After the embryos are recovered they are graded under a microscope as to stage of development and quality. Some ova are fertilized and progress to a developing embryo but then stop and become degenerate. Some ova are never fertilized and are called unfertilized embryos. This is the next biggest "X factor" in a MOET program. 80 to 90% of recovered embryos are transferable. A 70% pregnancy rate can be expected.

Risk of surgery: there is a probability of anesthetic deaths or other complications of surgery to the donors and the recipients. Most deaths are related to regurgitation during the procedure. It is critical that the animals are off feed and water 20 hours before surgery. This also makes the surgery easier to perform and hence a lot less of other possible complications that could come from surgery. Other complications can be hernias at the incision site, or adhesions of the uterus. Every measure should be taken to reduce any of these complications as much as it is possible. Surgical or laparoscopic methods are almost always used for collection and transfer. Ewe and doe recipients are synchronized so that ovulation occurs ~12 hr before donor ovulation. Transcervical collection of embryos in the doe has been attempted with variable success. Per rectum manipulation of the tract is not possible, so flushes generally involve the whole uterus rather than individual horns as in the cow. Although this method is possible in goats, it is much less repeatable in sheep because the more convoluted cervix is difficult to cannulate.

12.2.5.2. Synchronization and superovulation

Many combinations of treatments for the purposes of embryo collection and transfer are available. Estrus can be synchronized by the administration of progestagens such as progesterone

implants or synthetic progestins (flurogestone acetate, FGA; medroxyprogesterone acetate, MAP) given either orally or by the insertion of a vaginal sponge. The most widely used synchronization device for goats is the control internal drug release progesterone implant (CIDR-G, 0.3 g progesterone, Eazi Breed, InterAg, New Zealand) which is inserted in the goat's vagina using a special applicator. Most traditional schemes consist of a long progestagen (12–18 days) treatment; recent protocols use a shorter progestagen treatment (5–9 days) accompanied by a prostaglandin F2 α analogue injection. The method for synchronization of estrus is the same for both the donor and the recipients. Owing to the hormonal treatment administered to donors, estrus occurs sooner than in the recipients; therefore implants should be removed from recipients 12 hours prior to removal of the progesterone or progestin source from donors.

For the induction of superovulation of donor goats, pituitary extracts of follicle-stimulating hormone (FSH) and pregnant mare' serum gonadotropin (PMSG; also called equine chorionic gonadotropin, eCG) are the gonadotropins most used. Commercially available FSH products are: Folltropin V (Vetrepharm, Ontario, Canada), Ovagen (ICP, Auckland, NZ) and Super-Ov, (Ausa International, Tyler, TX). Several protocols can be used for superovulating goats, most commonly the injection of multiple doses of FSH on the last 3 to 4 days of the progestagen treatment. Due to the short half-life of the FSH molecule, it is traditionally administered every 12 hours. One example is the twice-a-day injection of a series of decreasing doses of FSH (5, 5; 3, 3; and 2, 2mg per injection), with a total dose of 20mg, with the next to last injection accompanied by progesterone removal and an injection of 150ug of a PGF2α analogue. Goats display behavioral estrus at approximately 24 hours after implant removal. Folltropin V can also be administered in decreasing doses (3.6; 3.6; 3.2; 3.2; 2.4; 2.4; and 1.6mg) with a 17-day progestagen treatment; a simpler protocol consists of six injections at a level dose given every 12 hours (total dose equivalent to 20 mg NIH-FSHP1).

When PMSG is used, a single injection 24 to 48 hours prior to implant removal is administered to the donor doe. Doses of PMSG range from 500 IU to 1500 IU, depending on breed, age, and size of doe and previous responses to treatment. One of the disadvantages of using PMSG is the tendency to produce persistent follicles that interfere with fertilization and overall embryo collection rates. It is important to note that there is a high variability in response rate to superovulatory treatments between animals, and factors such as follicular status, nutrition, lactation, seasonality, and management, among others, have an influence on the superovulatory response.

12.2.5.3. Estrus detection and breeding

Estrus is detected in donor does by observation of mating; in recipients estrus should be detected using vasectomized bucks or androgenized females. The time from beginning of estrus to ovulation in goats is approximately 32 to 36 hours. If fresh semen is being used, reports indicate that the effective dose is about 20×10^6 for laparoscopic AI, and 100×10^6 for cervical AI.

If laparoscopic artificial insemination is to be performed, does should be inseminated 12 and 24 hours after the animals were first seen in estrus or 45 to 50 hours after implant withdrawal.

12.2.5.4. Ova collection and transfer

Animals are taken off feed 18 to 24 hours prior to any surgical procedure. The animals are anesthetized with an anesthetic of choice. A combination of xylazine and ketamine (0.15mg/kg xylazine and 3.6mg/kg ketamine) is used intravenously to perform laparoscopic embryo transfer into recipients or for induction when gas anesthesia is used for embryo collection. When gas anesthesia is not available, a combination of xylazine and ketamine can be used alone (0.22mg/kg xylazine and 5.5mg/kg ketamine), with half the dose administered IV and half IM to maintain anesthesia during embryo collection. If necessary, animals can be re-dosed with 1.9mg/kg ketamine IV.

Animals are positioned in dorsal recumbence position with an inclination of 20 to 30 degrees with the head down. Exteriorization of the uterus through a midventral incision with or without examination of the ovaries should be made to determine the response to superovulation treatment. Embryo flushing medium is injected into the uterine horn and expressed out through a fluted plastic catheter inserted into the uterine tube through the fimbriated end. In the second procedure, the oviductal contents are flushed from the fimbria into the uterus and out of the uterus through a Foley catheter (size 8 Fr) inserted in the uterine horn near the bifurcation. This technique can be used for collecting embryos at any stage. In the third procedure, the uterine horn is flushed from the tip near the uterotubal junction toward a Foley catheter (8 Fr) inserted in the uterine horn near the bifurcation. This technique results in fewer adhesions because it eliminates the need to manipulate the uterine tube, fimbria, and ovaries. However, the technique is limited to use 4 or more days after estrus, at which time the embryos will have reached the uterus. Embryo collection rates using the surgical approach in goats average 85%.

12.2.5.5. Laparoscopic embryo collection

The donor animal is positioned in the laparoscopic cradle, and pneumoperitoneum is created. The primary trocar and cannula are inserted into the peritoneal cavity approximately 10cm cranial to the udder and 2 to 3cm to the left of the midline. Care should be taken to avoid major vessels under the skin and internal organs. The secondary trocar and cannula (5mm diameter) are inserted to the right of the midline. The laparoscope (5–7mm diameter) is inserted through the primary cannula and the uterus is located just cranial to the bladder. In some cases, the omentum or visceral fat may obscure the uterus. These can be pushed cranially with a manipulating rod placed through the secondary cannula. Once the uterus is located and the ovulation points counted and evaluated, a third cannula (5mm in diameter) is inserted on the midline 2cm cranial to the others. Through this third cannula, a long blunted paravertebral needle (12 gauge, 25cm long) is used to make a puncture wound in the uterine wall cranial to the bifurcation of the uterus, which is secured through the second cannula with atraumatic laparoscopic forceps. This allows the insertion of a balloon Foley catheter with stylet through the small puncture into the uterine lumen. The balloon on the catheter is inflated and the stylet removed. The uterus is secured by the inflated balloon. If a three-way catheter is used, atraumatic grasping forceps must be used to block the uterotubal junction to prevent flow of the medium and embryos toward the uterine tube. The flushing medium is introduced through the catheter and into the uterine horn by means of an inner extendable tube that reaches the uterotubal junction. Return of the flushing medium is initiated by pressure and gravity. Each horn is flushed with 30ml of medium, and

79% embryo recovery rates have been achieved. If a two-way catheter is used, the tip of the uterine horn is secured with atraumatic forceps and cannulated using an intravenous catheter (18 gauge, 51/2 inches long). After the stylet is removed, a collection medium (60ml) is introduced through the intravenous catheter and collected through the Foley catheter. The procedure is repeated for the other horn, the instruments are removed, and the abdominal incisions are closed. With this technique collection rates should be ranged from 50% to 80%.

12.2.5.6. Nonsurgical (transcervical) embryo collection

Nonsurgical (transcervical) embryo collection techniques avoid the formation of postsurgical adhesions and maintain the value of genetically superior donors following multiple embryo collections. The cervix may be primed using a combination of prostaglandins E2 and estradiol. The tents were inserted into the cervical canal for 6 to 12 hours and held in place by packing of the cranial vagina with gauze. The flushing procedures were performed in standing animals with a variety of catheters such as a modified two-way 24-gauge Foley catheter through which a stainless steel tube (50cm long, 1.5mm inner diameter) was inserted. A Sovereign catheter (5 Fr) was inserted through the stainless steel tube to introduce the medium. Other flushing cannulas were based on the model of the two-way catheter that was developed for use in cattle. The cannula was a concentric double tube with a metallic inner tube (Cathelin needle) for inflow of flushing medium and a polyethylene outer tube for outflow. Using this catheter, the practitioner flushed each uterine horn individually by pulling the catheter back into the uterine body prior to reinsertion into the other horn. Some of the problems encountered during these procedures were the incomplete passage of the cannula through the cervical lumen and the puncture of the uterine wall with the flushing device. Embryo recovery rates were 89.5% for 15 females.

12.2.5.7. Transfer of embryos

Transfer of embryos can be achieved surgically, laparoscopically, or nonsurgically to recipients that are detected in estrus at the same time as the donor doe, plus or minus 24 hours. Synchrony of donors and recipients is one of the most important factors in the success of an embryo transfer program. The number of embryos transferred per recipient is correlated with the survival of embryos. Studies indicate that there is a significant improvement when two embryos rather than one or three are transferred. Survival of embryos was also higher when unilateral transfer of both embryos was performed. The possible explanation is that there may be synergy between embryos in influencing each other's survival.

12.2.5.8. Surgical embryo transfer

The reproductive organs is approached through midventral laparotomy and exteriorized to allow visual confirmation of a CL prior to transfer of the embryos. Embryos are transferred to the uterine tubes via the fimbria using a tomcat catheter, Drummond pipette, or Pasteur pipette, if embryos are in the early stages of development (<day 4). Older embryos (>day 4) are transferred to the uterine horns using a Pasteur pipette or tomcat catheter through a small stab wound made with a rounded needle or with the eye of a suture needle.

12.2.5.9. Laparoscopic embryo transfer

Recipient animals are placed in a cradle angled to 40 degrees with the head down. Pneumoperitoneum is produced by introducing CO2 through a Verres needle inserted transabdominally. Two stab wounds are made in the abdominal wall, each 2 to 3cm from the midline and approximately 10cm cranial to the udder. One is for insertion of the laparoscope and the other for insertion of the laparoscopic grasping forceps. Using the grasping forceps, the terminal one half of the uterine horn ipsilateral to the CL is secured. Embryos designated for transfer are drawn into a 3.5-cm Fr tomcat catheter attached to a 1-ml syringe. For cannulation of the uterine horn, a 16-gauge 5.5-cm long Teflon catheter is inserted through the abdominal wall at the site directly ventral to the secured horn. The catheter is gently inserted into the uterine horn and upon penetrating the lumen, the stylet is withdrawn. The catheter is considered intraluminal when it is passed 2cm and can be manipulated back and forth freely. The Teflon catheter is removed and the tomcat catheter containing the embryos is guided through the cannula and into the lumen of the uterus. After the catheter can be freely maneuvered, the embryos are slowly expelled. The instruments are withdrawn and the incision sites on the abdominal wall sutured. An alternative technique uses the laparoscope for visualization of the ovulation site on the ovaries, and with the aid of Babcock forceps, a loop of the terminal half of the uterine horn ipsilateral to the CL is grasped and exteriorized through a 2-cm abdominal incision approximately 2cm from the linea alba (in place of the secondary trocar cannula). Once the section of uterine horn is outside the abdominal wall, a puncture wound is made through the uterine wall with a rounded needle (16 gauge). Through the same perforation, a tomcat catheter containing the embryos is introduced into the uterine lumen approximately 2.5cm toward the uterine body, and the embryos are deposited.

12.3. In vitro-fertilization

In vitro production of embryos (IVP) refers to the use of laboratory procedures to generate embryos for transfer, freezing, and other biotechnologies including cloning and transgenesis. In vitro production systems offer additional options to conventional ET for obtaining embryos from donor cattle of superior genetic merit. This technique includes collection of oocytes, in vitro fertilization, sexing of embryos and embryo transfer. In vitro fertilization may prove useful for obtaining large numbers of embryos for transfer.

12.3.1. In vitro-fertilization in camels

Dromedary embryos were produced in vitro using abattoir-derived oocytes, fresh (ejaculated) semen, and oviductal cell co-culture.

Experiments were conducted to study the effect of storing epididymal spermatozoa, in tris–tes- and tris–lactose egg yolk extenders, on their fertilizing ability and subsequent in vitro embryo development. Ovaries and testes were collected from a local slaughterhouse in normal saline solution

(NSS) at 37 °C and on ice (0–1 °C), respectively. Cumulus oocyte complexes (COCs) aspirated from the follicles were randomly distributed to 4-well culture plates (20–25 COCs/well) containing 500 µL of maturation medium and cultured at 38.5 °C in an atmosphere of 5% CO_2 in air for 36 h. Spermatozoa were collected from the cauda epididymides in syringes containing 2–3 mL of either tris–tes- or tris–lactose egg yolk extender. They were cooled down slowly and stored at refrigeration (4 °C) temperature. The spermatozoa were evaluated for motility and used for IVF of IVM oocytes on the day of collection and after 2, 4, 6 and 8 days of storage. On the day of IVF, spermatozoa were prepared by the swim up technique:

1. Transfer 1 mL of liquefied semen to a sterile round bottomed centrifuge tube. If the sample is too viscous, try diluting it with PureSperm®Buffer before.

2. Use a new pipette to carefully layer 1,5 mL PureSperm®Wash over the semen.

3. Without disturbing the layers, place the centrifuge tube at a 45° angle into a CO2 incubator, at 37°C for 60 minutes.

4. Carefully remove the uppermost (0,5-1,0 mL) of medium containing motile sperm using a sterile pipette.

5. Place this fluid in a sterile conical centrifuge tube containing 5 mL PureSperm®Wash.

6. Centrifuge at 500 x g for 10 minutes. Do not use the brake.

7. Aspirate the supernatant, leaving no more than 2 mm depth of liquid above pellet.

8. Resuspend the sperm pellet in a suitable volume of medium to obtain the required sperm concentration. The sample is now ready for analysis or use.

Both spermatozoa and oocytes were co-incubated at 38.5 °C in a humidified atmosphere of 5% CO_2 in air for 15–16 h. Presumptive zygotes were either fixed and stained with Hoechst 33342 for evaluation of fertilization or were cultured in 500 µL of the culture medium at 38.5 °C in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2 in air. There was no significant difference in the proportion of oocytes fertilized with spermatozoa stored in either of the two extenders for up to 8 days. The proportion of oocytes that cleaved (43–60%) and those that developed to blastocysts (14–21%) did not show any difference either, when spermatozoa from different days of storage were used. First cleavage was observed as early as 16 h after IVF, early blastocysts had developed by day 4, expanded blastocysts after day 5 and hatching of blastocysts started after day 6 of culture.

IVM/IVF procedures were conducted on six hundred and sixty four (664) cumulus oocytes complexes (COCs) aspirated from ovaries collected at a local slaughterhouse and cultured in vitro (38.5 ^{o}C; 5% CO2, and maximum humidity >95%). Maturation was completed by incubation in TCM-199 medium supplemented with 10% heat-treated Fetal Calf Serum (FCS), 10 ng/mL EGF, 1 µg/mL FSH, 1 µg/mL E2 and 500 µM cysteamine for 30 h. In vitro fertilization was performed using fresh semen (0.5 x 10^6 spermatozoa/mL in modified TALP-solution). Fertilized oocytes were cultured in mKSOMaa, under 38.5 ^{o}C, 5% CO2 and 90% N2 with maximum humidity (>95%). All IVC steps

were done in seven replicates. The cleavage rate (two cells to blastocyst stage) was 64% (425/664) and the percentage of oocytes reaching the blastocyst stage was 23% (155/664). The hatching rate of blastocyst obtained after culture was 46% (71/155). Good quality hatched blastocysts (n = 66) were transferred individually to synchronized recipients. Pregnancy rates, determined by ultrasonography at 15, 60 and 90 days after embryo transfer (ET), were 38%, 32% and 27%, respectively. Out of 18 pregnant females 5 aborted between the fifth and seventh month of pregnancy and 13 females (20%) remained pregnant. After 385 days of pregnancy, the first healthy and normal male-dromedary offspring produced fully in vitro was born at a birth weight of 38 kg. More dromedary calves (n = 4) were born later on. The remaining pregnant females (n = 8) were due to calf within the subsequent months.

12.3.2. In vitro-fertilization in horses

Whilst *in vitro* fertilization has been successfully performed in the mare, the technique is still in its infancy and has been significantly limited because: few oocytes can be collected at any one time; inducing sperm capacitation is complicated; oocyte penetration rates are low compared with some other species; and the technique requires synchronization of the donor with a number of potential recipients.

Several methods have been developed to collect oocytes, including (a) paralumbar needle puncture, (b) paralumbar laparotomy and (c) transvaginal ultrasound-guided aspiration. Usually only mature follicles are punctured, although, recently, oocytes have been recovered from follicles as small as 20 mm in diameter and follicles from mares in dioestrus and early pregnancy as well as mares in estrus.

12.3.1.1. Oocyte transfer

Rather than attempting to fertilize oocytes *in vitro*, an alternative method has been designed where oocytes are harvested from mature follicles of the donor mare and then transferred into the recipient mare.

- Follicle aspiration is performed on the donor as described above.
- The recipient's own oocyte is removed by follicle aspiration.
- Oocytes are transferred surgically into the uterine tube of the recipient (in some studies oocytes have been placed into the pre-ovulatory follicle of the recipient).
- The recipient is inseminated immediately before and after the transfer.
- Pregnancy rates can be as high as 50% after transfer (oocyte recovery rates are approximately 70%).
- Repeated follicle aspiration can be undertaken at successive cycles.
- The technique requires synchronization of the donor and a number of potential recipients.

Three experiments were conducted to evaluate the effect of oocyte and sperm treatments on rates of in vitro fertilization (IVF) in the horse and to determine the capacity of in vitro matured horse oocytes to be fertilized in vivo. There was no effect of duration of oocyte maturation (24 vs. 42 h) or calcium ionophore concentration during sperm capacitation (3 mM vs. 7.14 mM) on in vitro fertilization rates. Oocytes matured in 100% follicular fluid had significantly higher fertilization (13% to 24%) than did oocytes matured in maturation medium or in 20% follicular fluid (0% to 12%). There was no significant difference in fertilization rate among 3 sperm treatments utilizing 7.14 mM calcium ionophore (12% to 21%). Of in vitro-matured oocytes recovered 40–44 h after transfer to the uterine tubes of inseminated mares, 77% showed normal fertilization (2 pronuclei to normal cleavage). Cleavage to 2 or more cells was seen in 22% of oocytes matured in follicular fluid and 63% of oocytes matured in maturation medium; this difference was significant. It was conclude that in vitro-matured horse oocytes are capable of being fertilized at high rates in the appropriate environment and that in vitro maturation of oocytes in follicular fluid increases fertilization rate in vitro but reduces embryo development after fertilization in vivo. Further work is needed to determine the optimum environment for sperm capacitation and IVF in the horse.

12.3.3. In vitro-fertilization in cattle

The first calf resulting from the transfer of an embryo derived from an in vivo–matured oocyte, fertilized in vitro was reported by Brackett and colleagues in 1982. Currently, embryos are routinely produced in laboratories worldwide using a variety of embryo culture systems. In cattle, IVP typically consists of the following four components: retrieval of oocytes from ovarian follicles, in vitro maturation of oocytes (IVM), in vitro fertilization (IVF), and in vitro culture (IVC) of presumptive zygotes to the morula or blastocyst stage of development.

12.3.3.1. Oocyte recovery and transport

The first step in the IVP procedure is to obtain potentially competent oocytes from ovarian follicles of the donor cow. Ovarian follicles may be aspirated using laparoscopy, or more commonly by using ultrasound-guided. In cases of genetic salvage, the ovaries may be surgically removed using colpotomy in mature cows or by standard flank approach ovariectomy in heifers or cows. Oocytes can also be harvested from ovaries obtained at an abattoir. The skilled practitioner can learn to harvest oocytes from the ovaries of cows on the farm using ultrasound guided. Briefly, the cow is restrained in a squeeze chute, and the external genitalia and perineal area are cleaned. A standard epidural block of 2% lidocaine hydrochloride is administered to the donor cow, and a sedative may also be administered as needed. The transvaginal aspiration system consists of a good quality ultrasound unit fitted with a 7.5 MHz probe, a vacuum pump and regulator, a probe handle for housing the ultrasound probe, and aspiration needle. The hub of the aspiration needle is attached by tubing to an oocyte collection container, such as a 50ml conical centrifuge tube or an embryo collection filter. An additional piece of tubing connects the oocyte collection container to the vacuum pump. The aspiration needle and tubing

are first flushed with medium containing heparin. The probe handle containing the ultrasound probe and aspiration needle is then inserted into the vaginal vault. The operator's opposite hand stabilizes the ovary near the cranial vagina using per rectum palpation technique. As ovarian follicles are visualized on the ultrasound screen, the operator carefully advances the aspiration needle through the vaginal wall and pierces follicles to be aspirated with the needle. Follicular fluid and oocytes are aspirated into the collection tube or filter using a vacuum pressure of about 75mmHg. Oocyte retrieval can begin in cows at approximately 30 days post partum, a time when cows are not usually responsive to superovulation treatment. The procedure can be repeated as often as twice weekly until the desired number of oocytes is obtained from the cow. Each procedure takes approximately 30 minutes to perform. Oocyte yield is variable between donor cows; however, 4 to 6 oocytes suitable for IVP are commonly obtained from a healthy donor cow. Oocytes may be shipped in oocyte maturation medium held in a temperature-controlled portable incubator. Ovaries for shipment should be held in physiological saline penicillin in a sealed thermos bottle, and shipped in a cooler or Styrofoam box containing warm packs as needed. After arrival at the IVP facility, oocytes are aspirated from follicles and placed in oocyte maturation medium. Frozen semen to be used for IVP should be shipped to the facility either prior to or at the same time as shipment of the donor's oocytes or ovaries. Ideally, the semen should first be tested in the IVP system using oocytes obtained at a slaughterhouse.

12.3.3.2. In Vitro procedures and results

In the laboratory, immature oocytes with their cumulus cells (cumulus oocyte complexes, COC) are washed in modified Tyrodes medium (TL-Hepes), and matured for approximately 22 hours in vitro using tissue culture medium-199 (TCM199) with supplements. At the end of the maturation period, COC are placed in fertilization medium (TALP medium with supplements) with thawed frozen spermatozoa selected for high motility using swimup or Percoll sperm separation procedures. Gametes are co-incubated for 18 to 20 hours after which time presumptive zygotes are washed in TL-Hepes and placed into culture medium. Bovine embryos have been successfully cultured to the blastocyst stage using undefined media (commonly, TCM199 plus co-culture cells, serum, and other components), semidefined media (e.g., modified synthetic oviductal fluid, SOF with BSA), and fully defined media (e.g., SOF with polyvinyl pyrrolidone, PVP). The yield of transferable quality embryos after IVP varies from about 20% to 40% or greater. Embryo yield from cows in poor body condition, terminally ill, or infertile is often low and more unpredictable than that from healthy cows. The consistent production of good to excellent quality morulae and blastocysts from an IVP system requires meticulous attention to detail. A number of factors can influence the survival of embryos produced using in vitro systems including medium composition, atmosphere, oocyte quality, and embryo genotype. Acceptable pregnancy rates can be achieved following transfer of in vitro–produced embryos; however, these pregnancy rates are often lower than those seen after transfer of in vivo–produced embryos. Pregnancy rates of recipients following transfer of in vitro–produced embryos of grade 1 (good/excellent) were greater than those for embryos of grade 2 (46.9% versus 35.6%; 60% versus 46%). In addition to embryo quality, pregnancy rates after transfer of embryos produced in vitro are influenced by embryo culture medium, stage of embryo development, fresh versus frozen embryos, and synchrony of embryo development and recipient's day of the estrous cycle.

12.3.3.3 In vitro-fertilization in buffaloes

A study was conducted to improve *in vitro* maturation and cleavage rates of buffalo oocytes. Good quality oocytes were divided into two experiments. In Experiment 1 oocytes were cultured for 24 h in a CO2 incubator at 38.5oC either in TCM-199, Ham's F-10, MEM or FertiCult medium supplemented with either 10% FCS or 0.3% BSA. Experiment 2 was carried out to investigate the effect of different hormones (either 50 µg/ml eCG, 50 µg/ml FSH or 1 mg/ml E2) added to four of the afore mentioned media enriched with 10% FCS at the same culture conditions. Matured oocytes were fertilized *in vitro* using frozen thawed semen capacitated with heparin and caffeine. The sperm-oocytes were co-cultured for 22 h in BO or TALP medium. The fertilized oocytes were cultured in either BO or TALP medium for an additional five days at the same culture conditions and checked daily for cleavage. Addition of FCS to all media led to a higher maturation rate, without any significant variation, than BSA did (75.6 vs. 71.3%). Although no influence on the maturation rate was observed, addition of eCG to TCM-199, Ham's F-10 or MEM media resulted in a non-significant increase of *in vitro* maturation rate of buffalo oocytes compared to other hormonal additives to the same media. Furthermore, supplementation of maturation media with eCG resulted in a non-significant higher *in vitro* maturation rate of buffalo oocytes compared to FSH and E2 (80.4% for eCG supplemented media vs. 74.0% and 73.0% for FSH and E2, respectively). There was a non-significant difference in the cleavage rate of buffalo oocytes matured in TCM-199 supplemented with either sera or hormones and fertilized either in BO or TALP medium. However, the highest non-significant cleavage rate was achieved when oocytes were matured in TCM-199 supplemented with eCG and fertilized in TALP medium (50%). The overall cleavage rate was not significantly greater in TALP than in BO medium (33.7 vs. 15.5%). It could be concluded that supplementation of maturation media with FCS and/or eCG could successfully improve IVM rate of buffalo oocytes. Furthermore, high cleavage rate could be achieved when oocytes were matured in TCM-199 supplemented with FCS and eCG and fertilized in TALP medium. Cumulus-oocyte complexes were cultured for 24 h in either TCM-199 or Ham's F-10 with or without gonadotrophins and supplemented with either 20% buffalo oestrous serum (BES) or FCS. The maturation rates of oocytes cultured in TCM-199 or Ham's F-10 medium supplemented with 20% BES did not differ (47% vs. 44%). Addition of LH significantly improved the maturation rate in the Ham's F-10 medium supplemented with 20% BES. Fertilization rate was significantly improved when fresh ejaculated spermatozoa treated with 5 mmol caffeine and 10 pg heparin in BO medium (50%). Rate of cleavage and development were also higher when in vitro fertilization was carried out with fresh ejaculated spermatozoa treated with caffeine and heparin than with frozen-thawed spermatozoa. Development rate was enhanced when fertilized ova were cultured in ligated rabbit uterine tube. The results indicate that oocytes cultured in medium supplemented with BES and gonadotrophins reveal high rates of maturation and development to the blastocyst stage after fertilization with fresh ejaculated spermatozoa.

12.3.3.4. In vitro-fertilization in sheep and goats

A study was conducted to produce embryos from sheep ova collected from ovaries at the slaughterhouse using two different culture media. About 305 ovaries were collected from 153 ewes at the slaughterhouse of Riyadh City. More than 1000 ova obtained from them, they were classified

according to their morphological appearance into four groups. Only the ova with intact granulosa cells or filled cytoplasm (570 ova) were IVM in TCM-199 supplemented with FCS 10%, PMSG, LH and estrogen hormone After 29 hr., they were IVF with fresh diluted semen collected from a matured ram, in TALP medium. Only 57 (9.47%) of fertilized ova were cultured into two different culture media (TCM-199 from Sigma chemical company, and simple modified culture medium MCM). In the TCM-199, 22 ova (81.48%) cleaved into a two cell stage, 18 of them (66,66%) became as a four-cell stage embryo, 13 of them (48.14%) developed to an eight-cell stage, only 6 embryos (22.22%) became as a sixteen cell stage, and only 4 of them reached the 32-cell stage and more (morula stage). In the MCM, from the 27 fertilized ova 17 (62.96%) were cleaved to a two cell stage, 11 (40.47%) of them became as 4-cell stage embryo, only 7 (25.92%) cleaved to 8-cell stage, 4(14.81%) of them developed to 16-cell stage, and only 2 (7.4%) reached the 32-cell and more (the morula stage). It is concluded that the complex culture medium is better twice as the simple culture medium, and the development of sheep embryo did require some vitamin, and amino acids for their early development in vitro.

Experiments were carried out to achieve fertilization and initial embryonic development of goat oocytes in vitro. Oocyte/ cumulus complexes were recovered from large follicles (>7 mm) of hormonally treated does and from 1-6-mm follicles of ovaries from hormonally superstimulated and nontreated goats. Three different sperm treatment/IVF media were used: defined medium with modifications (mDM); TALP and HEPES-buffered M199 with modifications (mH-M199). Immature oocytes (from 1-6 mm, small antral follicles) were cultured for in vitro maturation in M199 buffered with bicarbonate and with modifications including supplementation with 20% (v/v) goat serum with either (a) 100 μg /ml, (b) 5 μg FSH/ml, or (c) no added gonadotropin control. Insemination of (in vivo or in vitro) matured oocytes was performed with swim-up separated and heparin-treated freshly ejaculated sperm; additionally, caffeine was included in the mDM treatment. Use of mDM yielded better results than mTALP or mHM199. Results with oocytes after IVM were significantly better than those obtained with oocytes matured in vivo (68.4% vs. 45.5%, $p < 0.05$). Presence of LH or FSH during oocyte maturation improved both the results over those of the control ($p < 0.05$). The highest proportion of fertilized oocytes (fertilization rate) was achieved by combining the use of mDM for sperm and *IVF* with IVM in the presence of LH. LH provided the highest proportion of inseminated oocytes that cleaved 39.5% vs. 23.3 when IVM was with FSH. For fertilization, mDM afforded the best results whether oocytes were matured in vivo or in vitro.

Effective activation protocols that can be used during nuclear transfer investigations in goats need to be developed. The development of IVF goat embryos with those of nonfertilized parthogenetically developing oocytes activated by treatment with either ionomycin or ethanol, both followed by immediate exposure to 6-diethylaminopurine (6-DMAP) were compared in one study. Cumulus oocyte complexes (COCs) recovered from abattoir goat ovaries were either matured in a conventional laboratory incubator or placed in pre-equilibrated maturation medium and shipped overnight in a battery-operated dry incubator to another laboratory. Mature COCs were allocated randomly to one of three treatment groups. Group 1 oocytes (n=169 shipped, n=253 not shipped) were fertilized in vitro at 24 h postmaturation (hpm). The remaining COCs were activated at 28 hpm in either ionomycin (Group 2: n=362 shipped, n=202 not shipped), or ethanol (Group 3: n=263 shipped, n=249 not shipped). Activated oocytes were immediately incubated in 6-DMAP for 4 h. Blastocyst

development was evaluated on Day 8 post-insemination/activation. Percent cleavage was comparable in shipped and nonshipped oocytes and in all treatment groups. In both shipped and nonshipped oocytes, parthenotes developing from ionomycin- and ethanol-activated oocytes had significantly greater blastocyst development ($P<0.01$) compared to IVF embryos (28.5 ± 3.0, 27.4 ± 2.8, 10.3 ± 3.0, respectively for the nonshipped oocytes and 9.9 ± 2.1, 10.3 ± 2.4, 3.7 ± 4.7 respectively for the shipped oocytes). Shipped oocytes had lower blastocyst development compared to nonshipped oocytes in the three treatment groups. The mean blastocyst cell number was not statistically different between shipped and nonshipped oocytes or among treatment groups, suggesting that all were equally viable.

12.4. Ovum Pick Up

In animals, oocytes have traditionally been obtained from follicle aspiration of slaughterhouse ovaries or via a flank laparotomy technique. Slaughterhouse material has the obvious disadvantage of lack of repeatability and there is often considerable delay in the time between oocyte collection and placement in culture medium. Surgical laparotomy techniques have obvious disadvantages in terms of ease and repeatability. Consequently, it was an important finding when Pieterse and his co-workers at Utrecht University, using cattle, were the first to describe the technique of oocyte aspiration during transvaginal ultrasound ovarian scanning in domestic animals. They coined the term 'ovum pick-up' (OPU) for the collection of the oocytes and OPU offered a repeatable, less invasive and less traumatic system to provide a source of oocytes. These oocytes could then be used in in-vitro maturation (IVM)/IVF programmes or gamete intra fallopian transfer (GIFT) studies.

12.4.1. Ovum pick up in camels

For *in vitro* embryo production, oocytes can be harvested from the ovaries collected from slaughterhouse or from the pre-ovulatory follicles of live animals by an ultrasound guided transvaginal ovum pick-up. The ultrasound guided transvaginal approach for collection of oocytes has been tried in llamas after superstimulation with either FSH or eCG. Studies were also conducted to apply the transvaginal ultrasound guided ovum pick-up (OPU) technique in dromedary camels after their ovarian super-stimulation and *in vivo* oocyte maturation. For collection of cumulus oocyte complexes (COCs) the transducer was guided through the vulva into the cranial most portion of the vagina and 17-gauge, 55 cm single-lumen needle was placed in the needle guide of the ultrasound probe and advanced through the vaginal fornix and into the follicle. Follicular fluid was aspirated using a regulated vacuum pump into tubes containing embryo-flushing media. Aspirates were searched for COCs using a stereomicroscope, and they were then denuded of cumulus cells by hyaluronidase and repeated pipetting. The oocytes were classified as mature (with a visible polar body), immature (with no visible polar body), activated (with divided or fragmented ooplasm) and others (degenerated and abnormal). Overall an average of 12.12 COCs was aspirated per animal with an oocyte recovery rate from the aspirated follicles of about 77%.

12.4.2. Ovum pick up in mares

Manipulating the equine ovary in vivo has many technical difficulties and this may be partly responsible for the generally low (20-30%) recovery rates found in the mare. Adequate preparation is essential to prevent movement and ensure adequate relaxation of the rectum. The most commonly used drug to achieve rectal relaxation is a hyoscine/dipyrone combination at a dose rate of 20 mg Hyoscine per 100 Kg body weight given intravenously 10 minutes prior to the aspiration procedure. Other groups have used atropine and propantheline bromide to achieve rectal relaxation. Following manual evacuation of faeces, 50 ml of 2% lidocaine infused into the rectum just before aspiration helps provide additional analgesia and relaxation. Sedation of the mare is also necessary and can be achieved by use of detomidine (Domosedan; Smith-KIine-Beecham) intravenously at a dose rate of 0.01 mg/kg given 5 minutes before starting. Concurrent administration of butorphanol (Torbugesic; C-Vet Ltd) helps provide additional analgesia. The mare should be restrained in a set of stocks/crush which limits her movement. It is particularly important to limit lateral movement as this can make fixation of the ovary against the ultrasound transducer difficult. After emptying the rectum, bandaging the tail and administering the intra-rectal local anaesthetic and the sedation and uterine relaxant, the vulva and perineal area are thoroughly cleaned and disinfected. The transducer within its holder is covered with parafilm to prevent vaginal mucus and other debris entering the opening of the needle guide and then sterile lubricant applied. The transducer is gently inserted as far as possible to the left (when puncturing the left ovary) or the right (when puncturing the right ovary) of the external Os of the cervix. By means of rectal manipulation, the ovary is positioned so that one or more follicle(s) are in the line of the needle by using the biopsy guide/puncture lines on the ultrasound monitor. On the instruction of the operator manipulating the ovary, the needle is then advanced beyond the needle guide, through the vaginal wall and into the follicle to be punctured. The instruction to advance the needle should only be given when the follicle has been steadily positioned on the puncture line. A distinct popping sensation is felt when the follicle is entered and the echoic needle can be visualised within the follicle. As soon as the tip of the needle has been seen to enter the follicle, suction is immediately applied and the follicle, as imaged on the ultrasound screen, begins to collapse. Suction should be continued until the follicle appears to have completely collapsed. If flushing of the collapsed follicle is being performed, the flushing fluid can be visualised on the ultrasound screen re-filling the follicle and confirming the needle is still located in the correct place. During and after aspiration of the follicle fluid, the needle should be slowly and gently rotated in an attempt to curette the follicle wall. In addition the follicle can be gently manipulated per rectum. This should help dislodge any oocyte tightly attached to the follicle wall. This is important in the mare as recent work has shown considerable differences in equine and bovine oocyte-cumulus morphology within the ovarian follicle including the presence of a 'thecal pad' in the mare which may at least partly explain the poorer recovery rates of the mare as compared the cow. In any case, it makes thorough aspiration of the follicle important in the mare. If a second follicle is seen adjacent to the punctured follicle, the direction of the transducer can be slightly adjusted so that the puncture line crosses the new follicle. Then the second follicle can be punctured and aspirated without withdrawing the needle from the ovary. This procedure should be repeated until all visible follicles have been aspirated. A 63% mean recovery rate for follicles punctured was reported during oestrus and this figure falls to 22% during diestrus. Others found no influence of stage of cycle on oocyte recovery rate.

12.4.3. Ovum pick up in cows

In cattle, different methods have been tried in order to retrieve oocytes from live animals. Of these techniques, the ultrasound-guided transvaginal follicular puncture technique, or ovum pick-up (OPU), has rapidly become an important part of the in vitro embryo production process for embryo transfers since it offers a possibility to recover oocytes from highly merited females, not only once, but repeatedly. The OPU technique comprises of several sub-procedures. The animals must be restrained to minimize the movement, and given an epidural injection as analgesia and also to relax rectum and vagina. An OPU device is then inserted into the vagina. The device contains an ultrasound probe and a puncture-needle system. Through a hand in the rectum, the ovaries are put into position and the needle is introduced into a follicle via the vaginal wall (puncture) and the content is aspirated. In total 1677 ova were collected from ten cows; 1342 (80%) were used for in vitro maturation, fertilization, and embryo culture. All ova were fertilized with semen from one bull, and 218 transferable embryos were produced. Calculated on a year basis, this would amount to 87 embryos per animal, with an intra-animal variation between 28 and 132.

12.4.4. Ovum pick up in buffalo-cows

The potential of the ovum pick-up technique was evaluated in 6 Italian Mediterranean buffalo cows. The cows were submitted to ovum pick-up twice weekly for 2 months. An additional 2-months cycle of ovum pick-up was performed in 3 of the buffalo-cows. The ovum pick-up sampling did not affect the resumption of reproductive activity of these animals. In fact, all the buffalo-cows conceived, on average, 47.5 ± 27.5 d after the last ovum pick-up. An average of 5.48 follicles was punctured, and 2.71 oocytes were collected per session. However, only 53.5% of these oocytes were suitable for in vitro embryo production. The number of punctured follicles differed between individual cows. There were no differences in the number of collected oocytes or in the recovery rates. The number of punctured follicles, the number of collected oocytes and the recovery rate were similar in the first and second months; the quality of the oocytes was, however, better in the second than in the first month. The increasing interval between 2 consecutive ovum pick-up sampling (intersession interval) caused an increase of the percentage of large follicles. Moreover, the increase of the intersession interval from 4 to 5 d decreased the quality of the collected oocytes. The efficiency of in vitro production of embryos to expanded blastocysts was 16.7%.

In the Thai swamp buffalo, oocytes were collected by ovum pick-up from six non-lactating multiparous swamp buffalo twice per week for 10 consecutive sessions followed by once-weekly collection for 10 consecutive sessions without hormone stimulation. In addition, oocytes were collected from slaughterhouse ovaries that were classified as follows: ovaries from non-pregnant cows with a visible corpus luteum (NPCL); pregnant cows with a corpus luteum (P); and non-pregnant cows without a corpus luteum (NP). Follicles in each group of ovaries were categorized as small (2–4 mm), medium-sized (5–8 mm) or large follicles (≥9 mm). The quality of the oocytes was assessed by their capacity to undergo *in vitro* maturation. The total number of observed follicles per session (all sizes combined) was larger in the once-weekly OPU group compared with the twice-weekly OPU group. In particular, the numbers of small and large follicles were higher in the once-weekly OPU group (5.2 ± 0.7 and 0.9 ± 0.2, respectively) than in the twice-weekly OPU group (3.9 ± 0.5 and 0.5 ± 0.1).

The number of medium-sized follicles did not differ between the groups. The percentages of oocytes with abnormal spindle morphology were not different between oocytes from the twice-weekly (30.0%) and the once-weekly (28.6%) OPU groups. A higher percentage of oocytes obtained *in vitro* (49.5%) exhibited nuclear abnormalities compared with those obtained *in vivo* (≤34.8%) after *in vitro* maturation. In conclusion, oocytes can be successfully collected by OPU in the swamp buffalo, without hormonal pretreatment, and per week more good-quality oocytes can be collected by twice-weekly OPU. In addition, oocytes collected from slaughterhouse ovaries can be used with the reproductive status of the cow having no influence on the maturation competence of oocytes.

12.4.5. Ovum pick up in ewes and does

In vivo ovum pick-up in sheep may be improved with a proper choice of aspiration elements (needle and tubing) and aspiration vacuum pressure. In an experimental study, visible follicles in ovaries of slaughtered ewes (treated separately according to their diameters: small <3 mm, medium 3-5 mm and large >5 mm) were aspirated using different combinations of the three studied factors such as aspiration flow rate (10, 20, 30, 40 and 50 ml water/min), needle gauge (18 and 20 G) and tubing inner diameter (1, 2 or 3 mm internal diameter). In Expt 2, a study with two 18 G needles of different lengths (18 G: 82 mm; 18 GL: 600 mm) was carried out, using ovaries obtained post-mortem, and performing in vivo laparoscopic follicular aspiration on ewes. Good quality oocytes were considered as those with both complete compact cumulus and a homogeneous cytoplasm. Recovery rate, proportion of good quality oocytes (good quality oocytes/100 oocytes recovered) and overall efficiency (good quality oocytes/100 follicles aspirated) were noted. In Expt 1, aspiration flow rate affect remarkable proportion of good quality oocytes (69.5%, 50.5%, 44.8%, 36.5% and 28.3% for flows from 10 to 50 ml/min respectively, $p<0.05$). Needle gauge did not affect aspiration device efficiency. Thin and intermediate tubings were more effective (overall efficiency rates: 34.9%, 32.3% and 28.1% for 1, 2 and 3 mm respectively). Follicle size did not affect recovery rate, but proportion of good quality oocytes was higher for large (77.9%) and medium (64.4%) follicles. Finally, some combinations of the aspiration device showed greater effectiveness. In Expt 2, needle length did not influence recovery rate, but good quality oocytes rate was significantly modified both post-mortem and in vivo (good quality rate for 18 G vs. 18 GL needles: 69.5% vs. 47.7% and 58.1% vs. 25.4%, post-mortem and in vivo respectively). It was concluded that low-aspiration flow rates (10 and 20 ml/min) with thin or intermediate tubings (1 and 2 mm), and any short needle (18 G or 20 G) are the most adequate aspiration factors for OPU in sheep.

Endoscopy is used for repeated ovum pick-up (OPU). In one study, 4 different treatment programs (Groups A, B, C and D) for repeated endoscopic OPU in sheep were investigated. The number of follicles and oocytes, quality of cumulus-oocyte-complexes (COCs), and detectable effects on fertility of the donor ewes were compared. Each group consisted of 5 East Friesian Milk sheep. In Group A, follicles were punctured twice a week, in Group B once a week, and in Group C once a week followed by administration of 1500 IU PMSG 48 h prior to OPU. In Group D follicles were punctured and the sheep stimulated with 1500 IU PMSG 48 h prior to OPU once every 2 weeks. The PMSG-stimulated sheep received anti-PMSG immediately after OPU. Over a period of 10 weeks 216 OPU-sessions were performed. A total of 1978 follicles were punctured, and 1098 oocytes were

recovered, for a collection rate of 55.5%. In the Groups A, B, C and D an average of 6.8, 8.6, 12.2 and 14.9 follicles per animal and session was aspirated, and an average of 3.8, 4.9, 7.0 and 7.6 COCs per animal per session was recovered, respectively. No significant differences between groups were observed in the collection rates (51.1 to 57.1%) or in the quality of the COCs, and 65 to 70% of the COCs were suitable for in vitro production of ovine embryos. Seven sheep developed small adhesions between the ovary and infundibulum. After the study, 15 ewes became pregnant following natural mating with the same fertile ram (5 from Group A, 1 from Group B, 4 from Group C and 5 from Group D). In conclusion, OPU once a week in PMSG/anti-PMSG treated ewes was found to be the most effective treatment program for oocyte collection.

In goats, the efficiency of laparoscopic ovum pick-up (LOPU) followed by in vitro embryo production (IVEP) in the propagation of aged goats with poor reproductive performance was evaluated. Follicular development was stimulated in donor goats with 80 mg follicle-stimulating hormone and 300 IU equine chorionic gonadotrophin administered 36 h before LOPU. In addition, goats were heat synchronized with intravaginal sponges containing 60 mg medroxyprogesterone acetate for 10 days and a luteolytic injection of 125 μg cloprostenol 36 h before sponge removal and LOPU. Following in vitro maturation (IVM), oocytes were fertilized in vitro with frozen-thawed semen produced using the egg yolk-free Bioxcell extender (IVM, L'Aigle, France). The average number of follicles aspirated, oocytes recovered and cleavage after IVM/IVF followed by a short 24-h in vitro culture in modified synthetic uterine tube fluid medium were17.9 +/- 8.0, 15.7 ± 8 and 72 ± 7% per goat, respectively. A total of 296 embryos were transferred into 50 heat-synchronized recipients, of which 40 became pregnant (80%) and 38 progressed all the way to term, delivering 86 live kids. It was indicated that LOPU-IVEP can be used successfully to extend the reproductive life of valuable goats that have acquired difficulties becoming pregnant by artificial insemination after multiple kiddings.

12.5. Cloning

Cloning is the creation of an organism that is an exact genetic copy of another. This means that every single bit of DNA is the same between the two. There are a couple of ways to do this:

1. Artificial Embryo Twinning

Artificial embryo twinning is the relatively low-tech version of cloning. As the name suggests, this technology mimics the natural process of creating identical twins. This is accomplished by manually separating a very early embryo into individual cells, and then allowing each cell to divide and develop on its own. The resulting embryos are placed into a surrogate mother, where they are carried to term and delivered. Again, since all the embryos came from the same zygote, they are genetically identical.

2. Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer (SCNT) uses a different approach than artificial embryo twinning, but it produces the same result: an exact clone, or genetic copy, of an individual. This was the method used to create Dolly the Sheep.

Somatic cell: A somatic cell is any cell in the body other than the two types of reproductive cells, sperm and egg. Sperm and egg are also called germ cells. In mammals, every somatic cell has two complete sets of chromosomes, whereas the germ cells only have one complete set.

Nuclear: The nucleus is like the cell's brain. It's an enclosed compartment that contains all the information that cells need to form an organism. This information comes in the form of DNA. It's the differences in our DNA that make each of us unique.

Transfer: Moving an object from one place to another.

To make Dolly, researchers isolated a **somatic cell** from an adult female sheep. Next, they **transferred** the **nucleus** from that cell to an egg cell from which the nucleus had been removed. After a couple of chemical tweaks, the egg cell, with its new nucleus, was behaving just like a freshly fertilized zygote. It developed into an embryo, which was implanted into a surrogate mother and carried to term.

The lamb, Dolly, was an exact genetic replica of the adult female sheep that donated the somatic cell nucleus to the egg. She was the first-ever mammal to be cloned from an adult somatic cell.

12.5.1. Cloning in camels

Injaz is the first cloned camel, 2005. The ovaries were removed and DNA extracted and placed in an egg taken from and re-implanted into the surrogate mother. In this study, somatic cell nuclear transfer was used to produce the first cloned camelids, a dromedary camel. Donor karyoplasts were obtained from adult skin fibroblasts, cumulus cells, or fetal fibroblasts, and in vivo-matured oocytes, obtained from preovulatory follicles of superstimulated female camels by transvaginal ultrasound guided ovum pick-up, were used as cytoplasts. Reconstructed embryos were cultured in vitro for 7 days up to the hatching/hatched blastocyst stage before they were transferred to synchronized recipients on Day 6 after ovulation. Pregnancies were achieved from the embryos reconstructed from all cell types, and a healthy calf, named Injaz, was born from the pregnancy by an embryo reconstructed with cumulus cells.

12.5.2. Cloning in horses

Researchers at Texas have achieved another cloning first with the successful delivery of a foal using oocytes from a live mare, the first such clone in the world. A mule named Idaho Gem was born after a normal 346-day gestation in the womb of a mare. The first created using oocytes from a live mare was born at the University of Florida, College of Veterinary Medicine (UF) in March.

12.5.3. Cloning in cattle

The SCNT is much more widely and efficiently practiced in cattle than in any other species, making this arguably the most important mammal cloned to date. While the initial objective behind cattle cloning was commercially driven-in particular to multiply genetically superior animals with desired phenotypic traits and to produce genetically modified animals-researchers have now started to use bovine SCNT as a tool to address diverse questions in developmental and cell biology.

12.5.4. Cloning in buffaloes

Cloned buffalo calf 'Garima,' has been reported in India. Scientists cloned Garima using tissue from a foetus as part of a "hand-guided cloning technique".

12.5.5. Cloning in sheep and goats

Dolly (5 July 1996 – 14 February 2003) was a female domestic sheep, and the first mammal to be cloned from an adult somatic cell, using the process of nuclear transfer. She was cloned by Ian Wilmut, Keith Campbell and colleagues at the Roslin Institute near Edinburgh in Scotland. Dolly was born 5 July 1996 to three mothers (one provided the egg; another DNA and a third carried the cloned embryo to term). She was created using the technique of somatic cell nuclear transfer, where the cell nucleus from an adult cell is transferred into an unfertilized oocyte (developing egg cell) that has had its nucleus removed. The hybrid cell is then stimulated to divide by an electric shock, and when it develops into a blastocyst it is implanted in a surrogate mother. Dolly was the first clone produced from a cell taken from an adult mammal. The production of Dolly showed that genes in the nucleus of such a mature differentiated somatic cell are still capable of reverting back to an embryonic totipotent state, creating a cell that can then go on to develop into any part of an animal. Dolly's existence was announced to the public on 22 February 1997.

Goat:

Massachusetts researchers have cloned three goats that are genetically altered to produce a protein in their milk. Iranian scientists have cloned a goat and plan future experiments they hope will lead to a treatment for stroke patients, the leader of the research said. The female goat, named Hana, was born in the city of Isfahan in central Iran.

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APPENDIX:Tables and Figures

Table 24: Characteristics of the semen ejaculate in farm animals

	Male camel	stallion	Bull	Buffalo-bull	Ram and Buck
Color	Milky – white - gray	Gary – white	White – creamy - yellow	White - creamy	White - creamy
Volume	5-8 ml	50-150 ml	3-8 ml	3-8 ml	0.5-2 ml
Sperm concentration	400,000 sperm/ul	120,000 sperm/ul	1,000,000 sperm/ul	1,000,000 sperm/ul	2,000,000 sperm/ul
Mass activity	absent	absent	++/+++	++	+++
Individual motility	55%	60%	75%	75%	90%
pH	8.6	7.4	6.4	6.4	6.8

Fig. 97: Semen collection by electrocejaculator in male camels (*Qassim-KSA, 2008*).

A) Teasing and stimulation of the stallion by keeping a female in estrus in the other side of the phantom.

B) The stimulated stallion is mounting the phantom while the operator hold the artificial vagina and the penis during ejaculation

C) Dismounting of the stallion after ejaculation and removing the artificial vagina

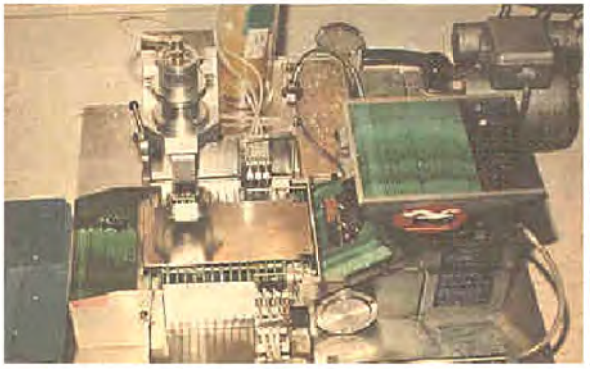
Fig. 98: Semen collection by artificial vagina in stallion (*www.equine.reproduction.com*).

A) parts of the artificial vagina

B)Technique of collection: the operator holding the artificial vagina in the right hand, the left hand is holding the prepuce and directing the penis toward the artificial vagina

C) Technique of electroejaculation: one operator holding the collecting funnel at the erected penis while the other keeping the probe of the electroejaculator inside the rectum

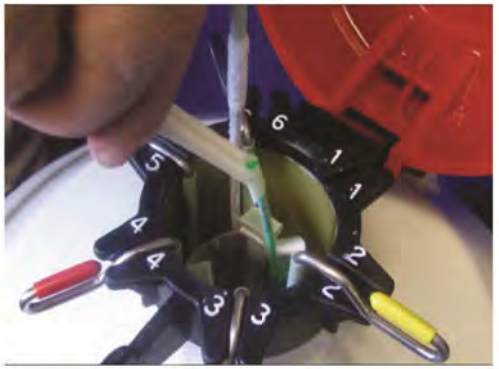
D) Preparation of the diluents for frozen semen



E) An automatic machine for filling, printing, and sealing of the straws

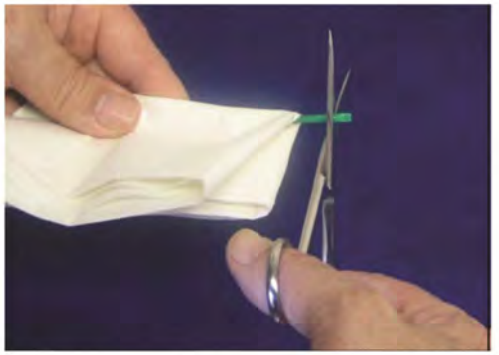
F) Liquid nitrogen tanks for cryopreserving semen (-196 °C).

Fig. 99: Semen collection and processing of frozen semen in bulls (*Berlin-Germany, 1999; Ohio-USA, 2005*).

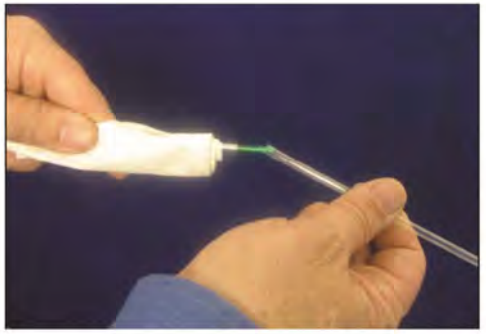


A) Taking the straw out the liquid nitrogen tank while the canister is kept down in the liquid nitrogen to avoid rising the temperature of the straws

B) Placing the straws directly into a container having worm water (40 °C/10 seconds or 39 °C/15 seconds)

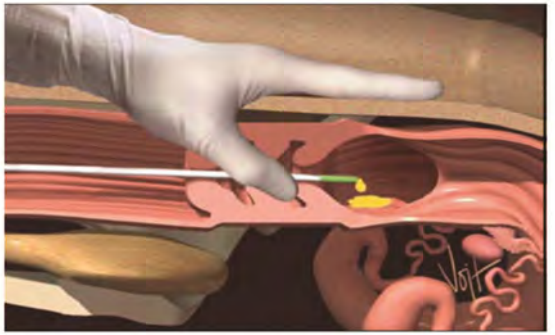


C) Dry the straw and cut the sealed end



D) Put the straw on the tip of the inseminating gun, then cover it by a plastic disposable inseminating pipette

E) Fix the inseminating pipette with a metal inesmeinating gun



F) Use the recto-vaginal technique to pass the inseminating gun through the vagina and cervix to the body of the uterus

Fig. 100: Technique of artificial insemination in cattle (*www.beefcattle101-wordpress.com*).

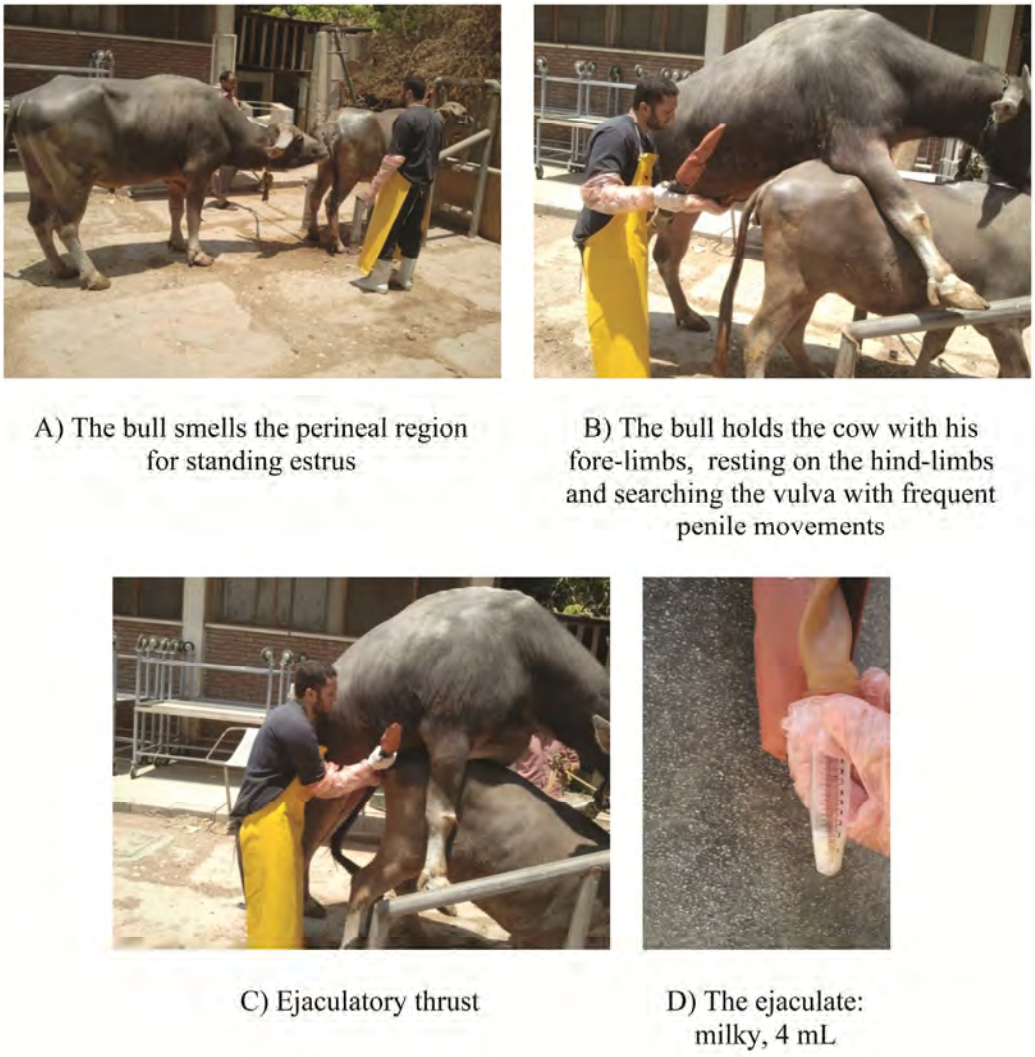
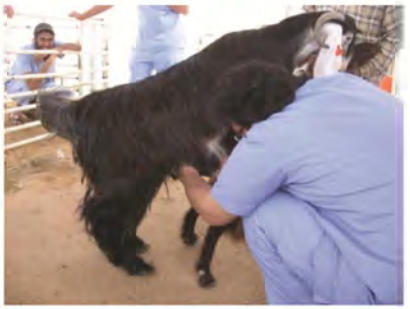
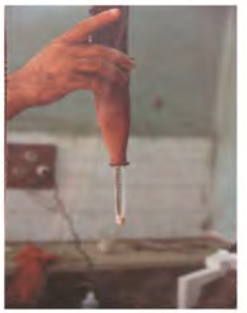


Fig. 101: Technique of semen collection in buffalo-bull (*Assiut-Egypt, 2013*).

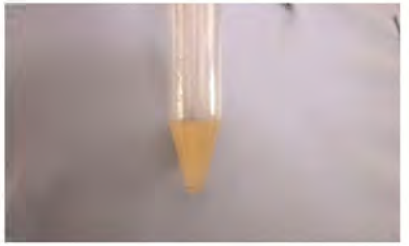
A) The buck tests the doe for standing estrus



B) The buck making a rapid mounting and ejaculation

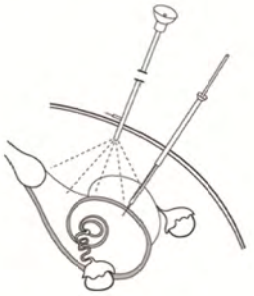


C) The ejaculate: creamy, 1.5 mL



D) Electroejaculation: the buck is kept in lateral recumbence, stimulated by the electroejaculation, and the ejaculate received in collecting funnel (yellowish, 2.5 mL)

E) Cervical insemination in a ewe.



F) Endoscopic insemination.

Fig. 102: Techniques of semen collection and insemination in small ruminants (*Assiut-Egypt; 2003; Qassim-KSA, 2008*).

A) Semen of camels: 6mL, creamy, viscous, of good concentration (400.000 sp/mm^3)

B) Semen of bulls: 9 mL, white (left tube) of high concentration (1.200.000 sp/mm^3)

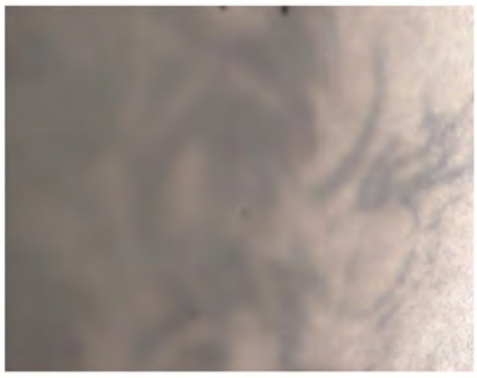
C) Semen of rams: 3 mL, opaque, of very high concentration (3.000.000 sp/mm^3)

D) Semen of an infertile camel: clear, had no sperms

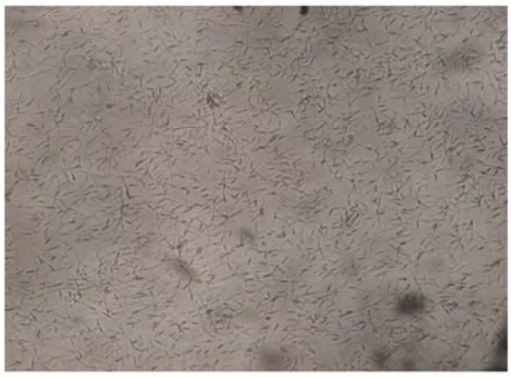
E) Semen of exhausted bull: clear (right tube) with low sperm concentration

F) Semen of infertile bull: red (with blood)

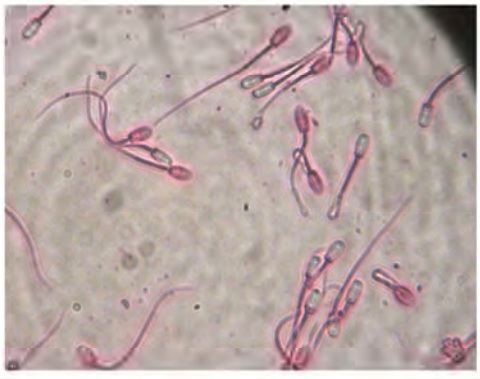
Fig. 103: Volume and color of semen in farm animals (*Assiut-Egypt, 2005; Qassim-KSA, 2009*).



A) Mass activity in rams (++)



B) Individual motility in rams (85%)



C) Eosin stain to differentiate between alive (colorless) and dead (red) sperms.

Fig. 104: Microscopic evaluation of the semen (*Qassim-KSA, 2009*).

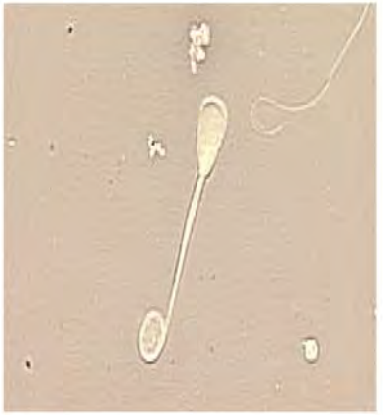
A) Narrow head (primary abnormality)

B) Kinked middle piece (primary abnormality)

C) Proximal protoplasmic droplet in immature sperm (secondary abnormality).

D) Distal protoplasmic droplet (secondary abnormality)

E) normal detached head (secondary abnormality)



F) Coiled tail (primary abnormality)

Fig. 105: Sperm abnormalities (*www.vivo.colostate.edu*).

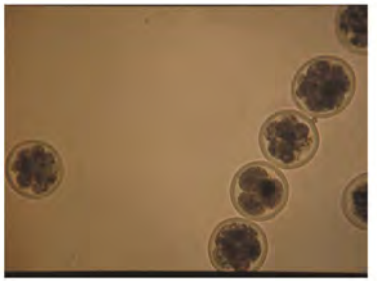
A) Preparation of media needed for embryo collection

B) Examination of the ovaries for the number of corpora lutea

C) Preparation of the Foley catheter and testing the balloon

D) Using the recto-vaginal technique to pass the Foley catheter through the cervix into the anterior third of the uterine horn

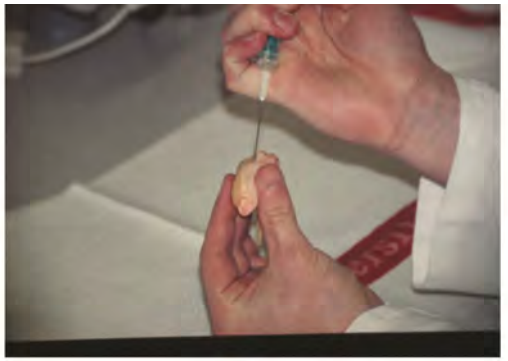
E) Searching the collected media for embryos



F) The collected embryos

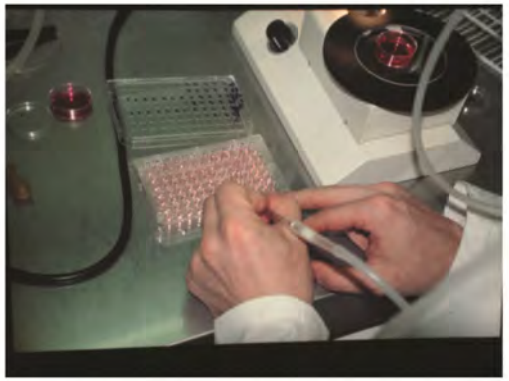
Fig. 106: Technique of embryo transfer in cattle (*Berlin-Germany, 1998*).

A) Collection of ovaries from slaughterhouses



B) Aspiration of follicles (2-8 mm in diameter) from the ovaries

C) Searching the collected fluid for immature oocytes

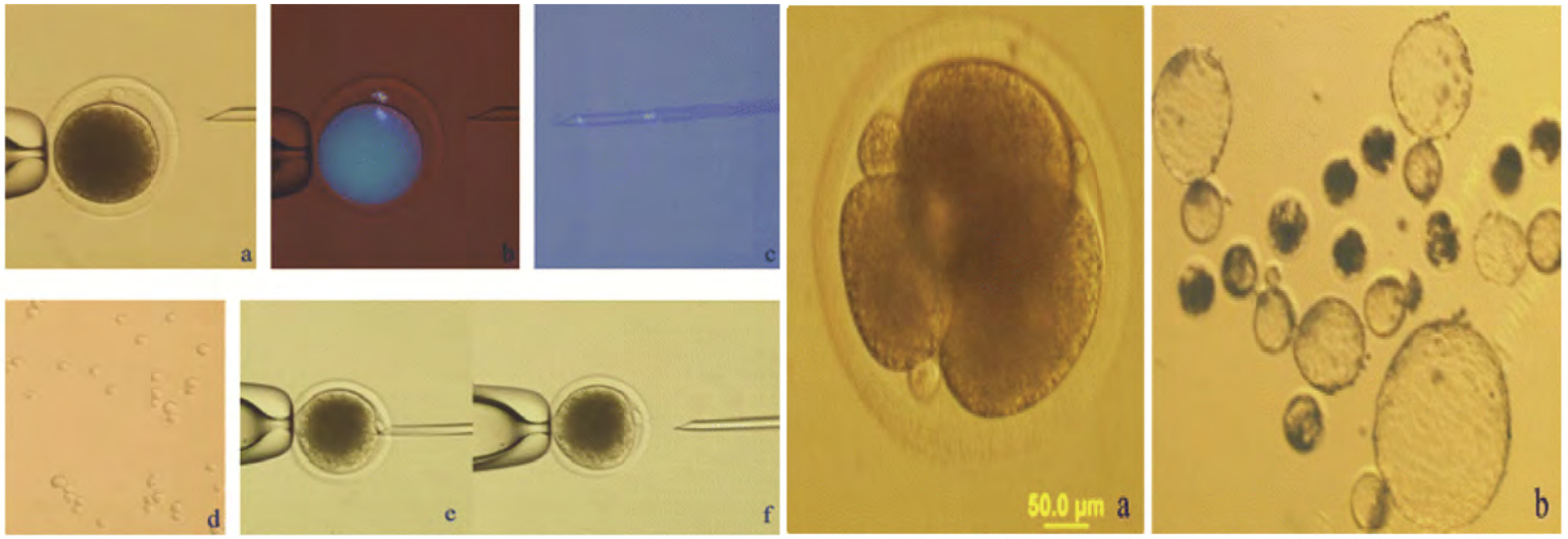


D) Placing 10 – 15 oocytes/ well containing 50uL tissue culture media

F) Putting the plate in a CO2 incubator at 39 °C for 24h

G) After 24h, examination of the oocytes for maturation, and use capacitated sperms for fertilization

Fig. 107: Steps of in-vitro fertilization (*Berlin-Germany, 1999*).



Steps in the somatic cell nuclear transfer (SCNT) of dromedary camel. **A**) A mature oocyte with a visible polar body held with a pipette. **B**) Determining the location of metaphase chromosomes by a very short (1–2 sec) exposure to UV light. **C**) Exposing the pipette to UV light to confirm the presence of both metaphase and polar body chromatin. **D**) Donor cells after trypsinization and washing. **E, f**) Injection of the donor cell into the perivitelline space of the enucleated oocyte. Original magnification ×200.

Development of SCNT embryos in dromedary camel: four-cell embryo on Day 2 (**a**) and blastocysts observed hatching out on Day 7 (**b**) of culture. Bar = 50 µm.

Fig. 108: Steps of SCNT and development of SCNT embryos in dromedary camels (*www.bioone.org*).

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