

<b>COURSE CODE:</b>	VCT 502
<b>COURSE TITLE:</b>	Veterinary Gynaecology
<b>NUMBER OF UNITS:</b>	3 Units
<b>COURSE DURATION:</b>	Three hours per week

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### COURSE DETAILS:

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### COURSE CONTENT:

Review of the structure and functions of the female reproductive organs and hormones. Puberty, physical and sexual maturity. Clinical evaluation of the female reproductive tract. Oestrus cycle, copulation, fertilization and gestation. Anoestrus and pseudo-pregnancy. Disturbances in ovulation and nidation. Infertility and sterility in females. Genetic and acquired anomalies of the reproductive tract and foetal development. Infectious and non-infectious diseases affecting sexual and reproductive functions. Diagnosis, treatment and prevention of mastitis.

### COURSE REQUIREMENTS:

This is a compulsory clinical course for all students undertaking the professional phase of the DVM programme. In view of this, students are expected to participate in all the course activities and have minimum of 75% attendance to be able to write the final examination.

## READING LIST:

1. Roberts, S.J. Veterinary Obstetrics and Genital Diseases. Ithaca New York. Edwards Brothers, 1971.
2. Nalbandov, A.V. Reproductive Physiology of Mammals and Birds. San Francisco. W.H. Freeman and Company. 1976.
3. Cole, H.H. and Cupps, P.T. Reproduction in Domestic Animals. New York. Academic Press. 1977.
4. Peters, A.R. and Ball, P.J.H. Reproduction in Cattle. Massachusetts. Blackwell Science. 1995.
5. Root Kustritz, M.V. Small Animal Theriogenology. Missouri. Elsevier Science. 2003.
6. Youngquist, R.S. and Threlfall, W.R. Current Therapy in Large Animal Theriogenology. Missouri. Saunders Elsevier. 2007.

## LECTURE NOTES

### INTRODUCTION TO THERIOGENOLOGY

Of all the mammalian bodily systems, reproduction plays a vital role in that it ensures perpetuation of the mammalian species through procreation. In the domestic livestock species, reproduction is wider in scope than actually imagined, thus, the term ‘theriogenology’ is often used to describe the complexity involved.

Theriogenology is a reproductive specialty practice providing expertise in animal reproduction. The practice offers routine management for the various animal species as well as specialized diagnostic and advanced reproductive techniques in these species.

Theriogenology is a term derived from the following Greek words:

<i>Therion</i>	-	beast
<i>Genan</i>	-	to produce
<i>Logus</i>	-	study of

Thus, **Theriogenology** can be defined as that branch of Veterinary Medicine which deals with reproduction, including the physiology and pathology of male and female

reproductive systems and the clinical practice of veterinary obstetrics, gynaecology and semenology.

**Veterinary Gynaecology** - that branch of medicine which treats diseases of the genital tract in the female.

**Veterinary Obstetrics** - It is that branch of veterinary science which deals with the necessary or advisable aid during the act of parturition in all animals coming under the purview of the veterinarian. It is the art of managing cases of animal birth. It is that branch of surgery which deals with the management of pregnancy, labour and *puerperium* (the period from the end of the 3<sup>rd</sup> stage of labour till completion of uterine involution).

Theriogenology has expanded to include all of veterinary reproduction including both male and female reproductive medicine, surgery, pathology and semenology. It encompasses companion animals, domestic livestock, exotic animals, zoo/wildlife and all avian species.

## **REVIEW OF THE ANATOMY OF FEMALE REPRODUCTIVE TRACT AND FUNCTIONS**

### **THE PELVIS AND PELVIMETRY**

The pelvis is the smallest and most posterior of the three great cavities of the trunk, and is of considerable importance to the obstetrician on account of its rigid wall, and the resulting resistance which it presents to the passage of the fetus as a result of any variation from the normal.

The attachment of the vulva to the pelvis constitutes the only fixed base of support for the vagina, cervix, and the uterus. This attachment enables the uterus at the time of parturition, to contract longitudinally in a manner which aids in dilating the birth canal and forcing the foetus towards the vulva.

Pathologically, the pelvis is of limited interest in veterinary obstetrics as compared with human obstetrics. The quadrupedal position of the domestic animal largely relieves the pelvis of weight-bearing, thus preventing deformities from pressure upon diseased pelvic bones, and as such renders pelvic deformities comparatively rare.

The pelvis is formed by both bony and ligamentous structures.

The bony pelvis is bounded at the sides and below by the two coxae and above by the sacrum and first three coccygeal vertebrae.

A. The *os coxae* each consists of an ilium, ischium and pubis. These paired bones form the chief boundary of the pelvis and the framework of the uppermost segment of the hindlimbs which it connects with the spine.

1. The **ilium** is the largest portion of the os coxarum and is irregularly triangular in shape. The ilia and acetabular part of the ischia form the lateral walls of the pelvis. The broad flat part of the ilium is called the wing, and the external portion of the wing is called the tuber coxae, hip bone or hook bone. The medial or pelvic surface of the ilia is smooth and is grooved for the obturator vessels and nerve.

2. The **ischium** is next in size to the ilium and forms the caudal part of the floor of the pelvis. The caudal lateral portion of the ischium is commonly referred to as the pin bone or tuber ischii. In the pig, the tuber ischii is mostly cartilaginous in nature and the symphysis does not undergo complete ankylosis until the animal is 6 to 7 years of age. The anterior border of the ischium forms the posterior boundary of the obturator foramen.

3. The **pubis** is the smallest of the three bones of the os coxae and forms the cranial portion of the floor of the pelvis. The dorsal or pelvic surface is smooth and usually concave in females while in males it may be convex. Occasionally in young heifers a sharp tuberosity projecting into the pelvic canal is present on the cranial portion of the pubic symphysis. This prominence may rarely cause contusion or even laceration of the birth canal during difficult birth. The cranial medial border of the pubic bone provides attachment for the prepubic tendon while the caudal border forms the cranial boundary for the obturator foramen.

The acetabulum is formed by fusion of the ilium, ischium, and pubis. These bones form a cotyloid cavity in which the head of the femur lodges.

The round ligament is a short, strong band between the head of the femur and the acetabulum.

B. The **Sacrum**: Consists of a series of five fused vertebra in the horse and ruminants, four in the pig, and three in carnivora. It is somewhat triangular in form with the base

articulating anteriorly with the last lumbar vertebra and caudally with the first coccygeal vertebra with which it may some time fuse in old cows, horses and pigs.

The ventral or inferior surface of the sacrum is smooth and concave and forms the largest portion of the roof of the pelvis.

C. The ligamentous portion of the pelvis consists of:

(i) The dorsal and lateral sacro-iliac ligaments are attached to the medial wing of the ilium and the lateral portion of the sacrum and summits of the sacral spines.

(ii) The sacro-sciatic ligament completes the lateral wall of the pelvic cavity behind and between the sacrum and coxae. This ligament extends from the lateral border of the sacrum and the transverse processes of the first two coccygeal vertebrae to the ischiatic spine and tuber ischii.

Functions of the Sacro-Sciatic Ligaments:

1. They furnish attachments for the vulva and gluteal muscles
2. They prevent the posterior portion of the pelvis or ischia from rescinding from the sacrum.
3. They form a strong, flexible, and yielding wall to the pelvic cavity.
4. Under pressure of parturition, they yield sufficiently to allow the constricted pelvic outlet to equal in dimensions the larger bony inlet of the pelvis.

D. The prepubic tendon is essentially the tendon of insertion of the two *rectii abdominis* muscles but also furnishes attachment for the external and internal obliques the gracillis and pectini muscles. It is attached to the anterior borders of the pubic bones. The prepubic tendon prevents the pubis and ischium from passing upwards and backwards toward the sacrum when the body weight is placed upon the coxo-femoral articulation. The prepubic tendon is important in *herbivora*, especially in the mare, where it sometimes rupture during advanced pregnancy resulting in a hernia of the gravid uterus and a destruction of the normal relationship of the pelvis to the spinal column.

Rupture of the prepubic tendon is not too common in the cow. Herniation of the gravid uterus in the bovine are most commonly due to rupture of the abdominal muscles.

### **Articulation of the Pelvis**

There are five (5) pelvic articulations:

1. Sacro-lumbar
2. Right sacro-iliac
3. Ischio-pubic symphysis
4. Sacro-coccygeal
5. Left sacro-iliac

These articulations are important in obstetrics because they become flexible at the time of parturition.

The pelvic cavity in the domestic animals is somewhat conically shaped with the base of the cone located cranially.

The pelvic inlet is bounded by the following:

1. Dorsally the base of the sacrum
2. Ventrally the pelvic brim
3. Laterally the iliopectineal line.

In all species, the pelvic inlet is roughly oval in shape with the sacro-pubic diameter being the largest. The sow and cow have the most elliptical pelvic inlets while the mare and some dogs have inlets that are nearly rounded.

The pelvic outlet is bounded by:

1. The sacrum and first three coccygeal vertebrae dorsally
2. The ischial arch ventrally
3. The posterior-border of the sacro-sciatic ligament laterally.

The caudal portion of the pelvic cavity is smaller in diameter than the cranial portion, however it is capable of dilating at the time of parturition to allow the passage of the foetus. This ability to dilate is brought about by the relaxation of the pelvic ligaments, especially the sacro-sciatic ligament.

The pelvis of the male domestic animal differs from the female in a number of definite points.

1. The diameter of the pelvic inlet is usually smaller in the male than in the female.
2. The bones in males are thicker and heavier than in females

3. The ischial arch is usually narrower in males
4. The obturator foramen is smaller in males
5. The cranial floor of the pelvis is likely to be convex in the male while in the female it is usually concave.

The coxo-femoral articulation or hip joint is a ball and socket joint with the head of the femur fitting into the acetabular fossa which is made deeper by the cotyloid ligament - a fibrous band circling the acetabular fossa. The transverse ligament is that portion of the cotyloid ligament which crosses the acetabular notch. The round ligament extends from the subpubic groove in the acetabulum to the head of the femur and is intra-articular. In occasional cases this ligament may be small or absent. The accessory ligament, found only in the horse, extends from the prepubic tendon to the head of the femur.

A very common condition encountered in the cow is coxo femoral luxation or as it is commonly called "dislocation of the hip". The increase in the frequency of this condition in the bovine is due to:

1. Shallowness of the acetabulum
2. Lack of bulky muscles around the joint
3. The small or occasionally absent round ligament
4. Absence of the accessory ligament
5. Excessive relaxation of the pelvic ligaments in advanced pregnancy and in animals with cystic ovaries
6. The large size and weight of the abdomen in advanced pregnancy and in dropsy conditions of the foetal membranes, e.g. *hydrops allantois*.
7. The awkward gait

#### **PELVIMETRY**

The term pelvimetry refers to the measurement of the dimensions of the pelvic cavity. In human, obstetrics pelvimetry has been a routine part of the prenatal management of the expectant mother to determine ease of delivery. This procedure was given very little attention in veterinary obstetrics until the last decade. At the present time there is a great deal of interest in pelvimetry and the information obtained is being utilized to reduce the incidence of dystocia in heifers of beef breed.

Studies carried out in Nebraska, Montana and Colorado have disclosed that the major cause of dystocia in 2-year-old heifers of beef breeds is a disproportionately large calf at birth in relation to the dam's birth canal (pelvic size). It was noted that heifers with a small pelvic area had twice the incidence of dystocia as those with above average pelvic area (49% vs. 24%). It was also noted that pelvic area is moderately to highly heritable (above 50%) and can be increased in a herd through selection of breeding heifers and breeding bulls.

The ideal time for predicting dystocia in heifers is before they are bred as yearlings. Research has shown that yearling pelvic size is the most reliable yearling factor indicating potential difficulty since pelvic area growth was linear from 9 to 24 months of age in heifers calving at 2 years of age.

Studies on pelvic area: birth weight ratio has shown that a 600 pound yearling heifer with a pelvic area of 140 sq.cm. can usually deliver a 70 pound calf without major difficulty as a 2 year old. This pelvic area to birth weight ratio is 2 to 1. As the ratio decreases, the degree of calving difficulty increases and a caesarean section would generally be required with a pelvic area of 120 sq. cm. and a calf birth weight of 80 pounds which yields a ratio of 1.5 to 1.

Yearling pelvic areas (heifer weight 550-700 lbs) can be divided by a factor of 2 to estimate the calf birth weight a heifer can deliver as a 2-year old. If pelvic measurements are taken at the time of pregnancy examination (18 months old, weight 700-850 lbs.) a factor of 2.5 can be used to estimate calf birth weight. A factor of 3.1 can be used on pelvic areas taken before calving (2 year old weight 800 to 950 pounds). If yearling heifers weigh 750 to 850 pounds, a factor of 2.25 should be used. These factors appear to be good indicators of dystocia, with an accuracy of about 80%. Average pelvic area growth has been calculated to be 0.27 sq cm /day, from yearling to 2- year old heifers. If pelvic measurements are obtained before breeding, problem heifers with a small pelvic size can be culled from the herd or mated to easy-calving bulls, while heifers with a large pelvic diameter can be mated to average calving bulls. Since larger heavier heifers do not always have the largest pelvic size, heifers should be measured and mated according to pelvic size.

If heifers are measured at the time of pregnancy examination, problem heifers can be culled or aborted and sold as feeders. Heifers bred to produce a calf larger than can be delivered through their birth canal can also be marked for close observation at calving.

There are two methods used in determining the dimensions of the pelvic cavity:

1. **External or indirect pelvimetry** is carried out by calculating the pelvic diameter based on measurements taken between the angles of the ilia, the distance between the ischia and the height from the hip joint to highest point of the croup. In general, this method has been shown to be inaccurate and is no longer used.
2. **Internal or direct pelvimetry** refers to the measurement of the interior of the pelvis through the rectum with a **pelvimeter**.

#### **PROCEDURE**

The general procedure is to restrain the animal in a chute with light squeeze. A comfortable normal standing position is the best. Faeces should be removed from the rectum and the instrument carefully inserted into the rectum with the hand and arm to the pelvic inlet.

The width of the pelvic inlet is obtained at its widest point between the right and left shafts of the ilium. This is the horizontal (bisiliac) diameter of the pelvis.

The height of the pelvic inlet is obtained by measuring the distance between the dorsal pubic tubercle on the floor of the pelvic and the sacrum, dorsad. This is the vertical (sacro-pubic) diameter of the pelvis. The two measurements are read in centimeters and are multiplied to give the pelvic area in square centimeters.

#### **FEMALE REPRODUCTIVE SYSTEM**

The female reproductive system consists of the ovaries and the female duct system. The duct system includes the oviducts (fallopian tube/uterine tubes), uterus, cervix, vagina and vulva. The ovaries originated from the secondary sex cords of the genital ridge while the duct system arose from the mullerian ducts (a pair) which appear during early embryonic development.

##### **3.1.1 THE OVARIES**

They are considered the primary reproductive organs in the female because they produce the female gamete (the ovum) and the female sex hormones (oestrogen and progesterone). Thus, the ovary is both an endocrine and exocrine organ. The ovary is located in the abdominal cavity and is covered by a surface epithelium that is cuboidal which transforms into squamous with age advancement. The surface epithelium is usually referred to as “Germinal epithelium”.

The ovary consists of two zones: outer cortex and inner medulla (*zona vasculosa*). The cortex contains numerous follicles which are in various stages of development as well as corpora lutea, interstitial cells and stroma elements.

The cortex occupies the greater part of the ovary and is the target point for somatotrophic hormones like LH and FSH. The production of ovarian hormones in response to the tropic hormones is responsible for the cyclic activity of the ovary.

The medulla is composed primarily of blood vessel, nerves and connective tissues.

The germ cells arise from the embryonic gut tissue and then migrate to the cortex of the embryonic gonad. Just beneath the surface epithelium is a thin, dense layer of the connective tissue, the tunica albuginea ovarii which has a parenchyma below it which contains ovarian follicles and the cells that produce ovarian hormones.

Primary follicles are formed during the prenatal period of the female. Primary follicle is a germ cell surrounded by a single layer of granulosa cells. They are located in the parenchyma and frequently seen in groups called egg nests. Follicles are in a constant state of growth and maturation.

The stage of Primary follicle is followed by a proliferation of granulosa cells surrounding the potential ovum. And a potential ovum surrounded by two or more layer of granulosa cells is secondary follicle. Later in the development, an antrum (cavity) is formed by fluid collecting between the granulosa cells and separating them. With antrum formation, the follicle is classified as a tertiary follicle which later matures and appears as a fluid-filled blister on the ovarian surface called Graafian follicle. The fluid in the antrum is called "Liquor folliculi". It is a viscous fluid that is rich in steroid reproductive hormones particularly oestrogens.

Several cell layers occur in the Graafian follicle – the outer, more fibrous is the theca externa and the inner, theca interna-which secretes oestrogen.

Primary oocytes that are found in the ovarian cortex are large round cells with a vesicular nucleus and prominent nucleolus.

The granulosa cells that immediately surround the ovum is *cumulus oophorus* while those adjacent to it are called *corona radiata*.

The Graafian follicle is a large structure with very large antrum which protrudes at the ovarian surface and can extend to the depth of the cortex. Ovulation is achieved by a rupture of the matured follicle which leads to the extrusion of the ovum into the infundibulum of the oviduct.

In case of degeneration of the follicle in the advanced stage, it results in the formation of a scar, the *corpus atreticum* (atresia) consequent upon sudden disintegration of cells with invasion of blood etc. With rupture of the follicle, bleeding occurs and a blood clot forms at the ovulation site. The ruptured follicle with its blood-filled cavity, a *corpus haemorrhagicum*, is replaced by the *corpus luteum (CL)*, which forms rapidly by proliferation of a mixture of theca externa, theca interna and granulosa cells. CL is a solid, yellow body which is the only source of progesterone.

The corpus luteum is formed by rapid proliferation and hypertrophy of the granulosa cells in conjunction with the accumulation of a liquid pigment (lutein) within the cells.

If fertilization does not occur, the corpus luteum regresses and become fibrotic, thereby losing its yellowish colour and appearing as a small white scar on the ovarian surface called *Corpus albicans (C. fibrosum)*.

### **OVIDUCTS (FALLOPIAN TUBES)**

The oviducts are a pair of convoluted tubes extending from near the ovaries to and becoming continuous with the tips of the uterine horns. They function in the transport of ova and spermatozoa, which must be conveyed in opposite directions. In addition, they are the site of fertilization and the early cell division of the embryo.

Histologically, they contain three distinct cell layers, viz:

- The outer layer, basically connective tissue – *tunica serosa*
- The middle layer, composed of both circular and longitudinal smooth muscle fibres – *tunica muscularis*
- The inner layer, which contains both ciliated and secretory epithelial cells – *tunica mucosa*

An oviduct is divided into three segments i.e.

- Infundibulum (funnel-shaped): it has numerous mucosal folds and mucosal cells are ciliated.
- Ampulla which accounts for about half of the total length of the oviduct. It has longitudinal mucosal lining which greatly increase the surface area of the lumen.
- The Isthmus: it has a higher ratio of secretory to ciliated cells and distinct thicker smooth muscle layer than the ampulla. It joins the tip of the uterine horn at the uterotubal junction.

Generally, oviductal activity is stimulated by oestrogen and inhibited by progestins.

### **UTERUS**

Uterus extends from the uterotubal junctions to the cervix. The major function of the uterus is to retain and nourish the embryo or foetus.

Four basic types of uteri are found in animals viz:

- |               |               |
|---------------|---------------|
| i. Bicornuate | iii. Duplex   |
| ii. Simple    | iv. Bipartite |

Only two of these are found in farm animals i.e. i and ii above.

The tunica serosa is the outer layer of the uterus while myometrium is the middle layer composed of two thin longitudinal layers of smooth muscle with thicker circular layer sandwiched between. The endometrium is the mucosal lining which has simple glands. It responds to stimulation by oestrogen and progestins.

The endometrium provides a mechanism for attachment of the extra-embryonic membranes. This union forms the placenta through the process called **PLACENTATION** which aids in the transport of nutrients from the maternal blood to embryonic or foetal blood as well as waste product removal from the foetal blood through the maternal systems.

The nature of the placental attachments differs among species.

Chorionic villi from the extra-embryonic membranes penetrate into caruncles which are button-like projections on the endometrium. This union forms the **PLACENTOME**.

### **CERVIX**

It is thick-walled and inelastic, the anterior end being continuous with the uterine body while the posterior end protrudes into the vagina. For most farm species, the length will range from 5 to 10cm with an outside diameter of 2 to 5cm. It contains a canal which is the opening into the uterus. The primary function is to prevent microbial contamination of the uterus. It also may serve as a sperm reservoir after mating.

Histologically, the outer layer of the cervix is the tunica serosa. The middle layer is connective tissue interspersed with smooth muscle fibres that gives it the firm and inelastic properties. The inner layer, the mucosa is composed mainly of secretory epithelial cells with some ciliated epithelial cells present. High levels of oestrogens cause the cervical canal to dilate during oestrus. Synergism between high levels of oestrogens and relaxin causes dilation just before parturition.

### **VAGINA**

It is tubular in shape, thin-walled and quite elastic. It is the female organ of copulation. The outer layer, the tunica serosa, is followed by a smooth muscle layer containing both circular and longitudinal fibres. The epithelium is stratified squamous usually non-glandular and non-keratinized. The epithelial cells cornify under the lubricating or protective mechanism which prevents abrasions during copulation. Under the influence of progestins, the epithelial lining regenerates.

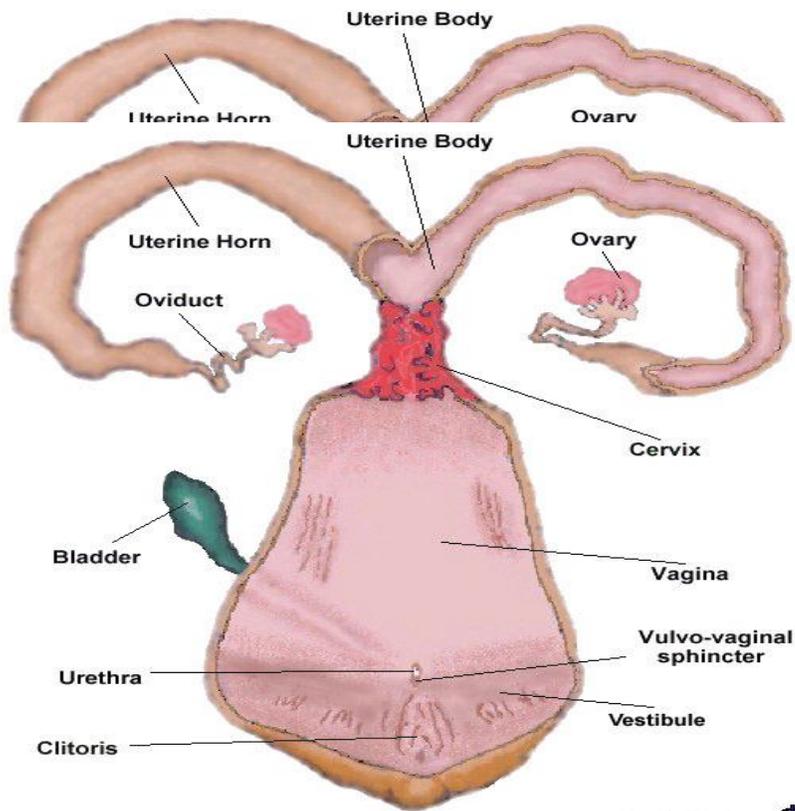
### **VULVA**

The vulva or external genitalia consist of the vestibule with related parts and the labia. The vestibule is the portion of the female duct system that is common to both the reproductive and urinary systems. The vestibule joins the vagina at the external urethral orifice. A sub urethral diverticulum is located just posterior to the external urethral orifice.

The labia close the vestibule. The clitoris, homologous to the *glans penis* in the male, is located ventrally and about 1 cm inside the labia. It contains erectile tissue and well supplied with sensory nerves.

## Female Reproductive Tract

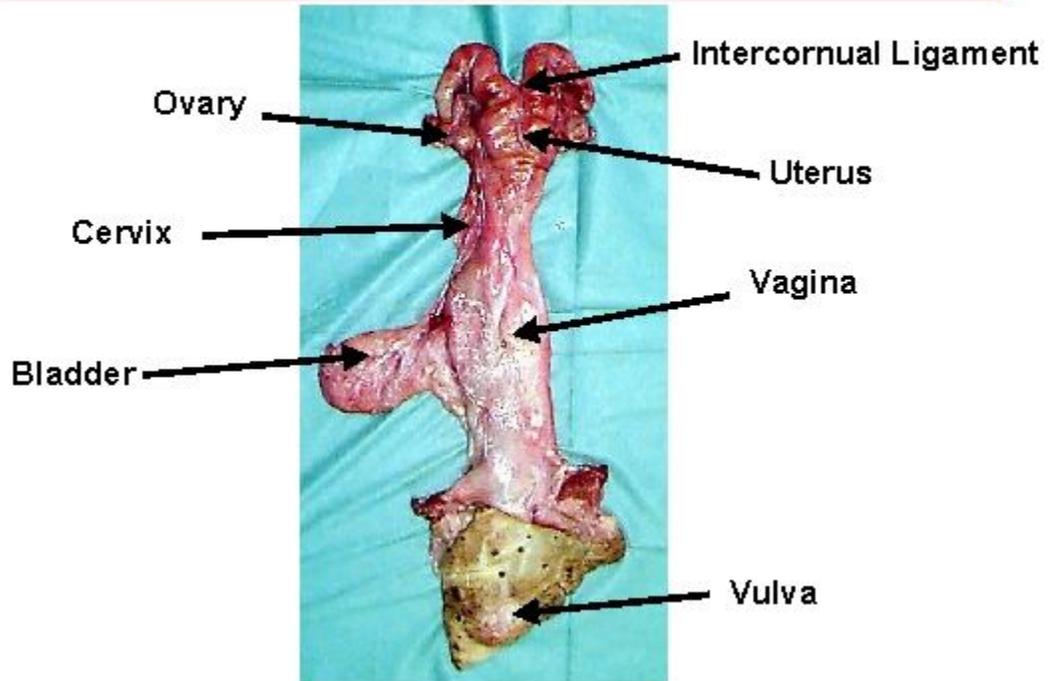
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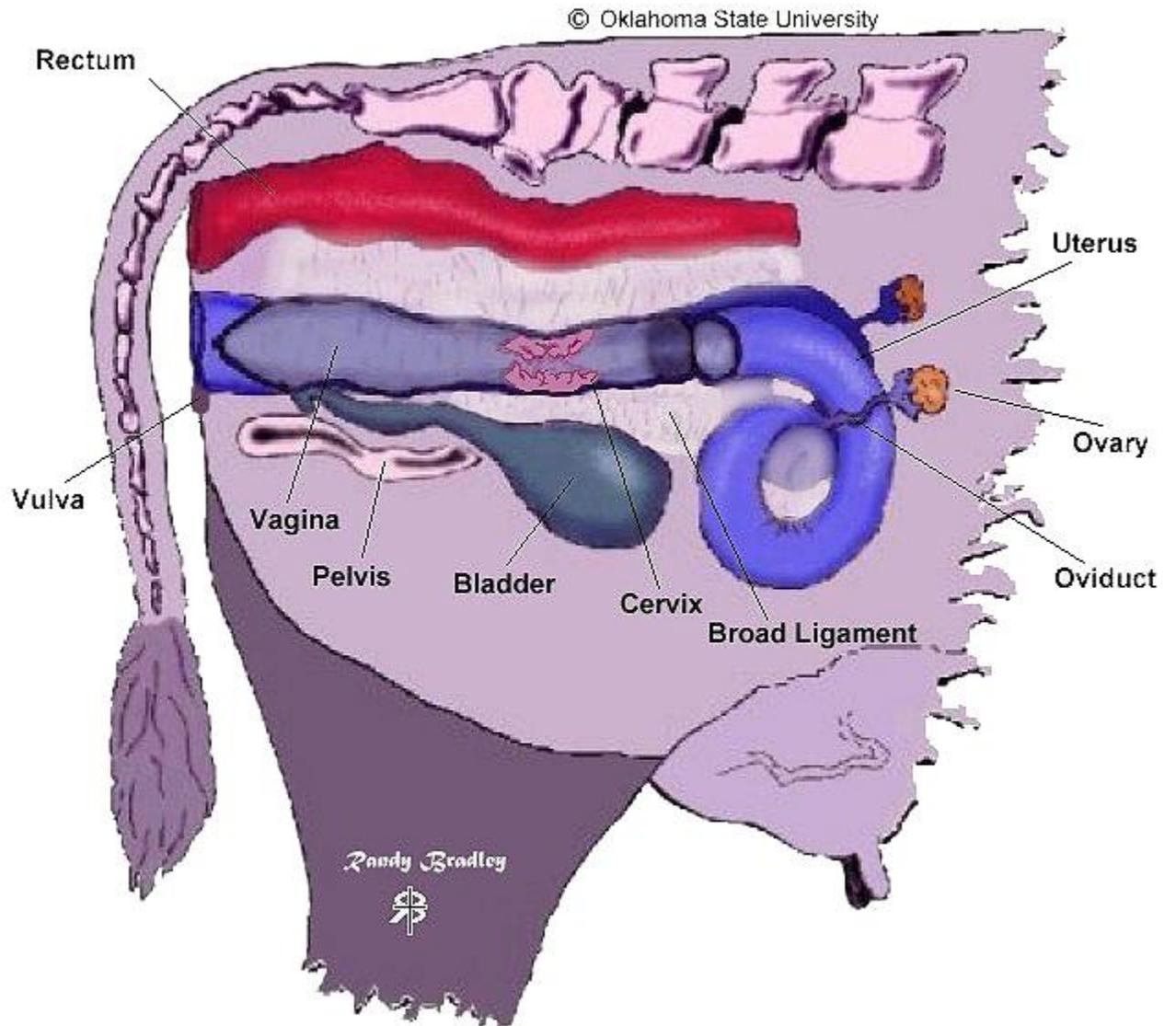


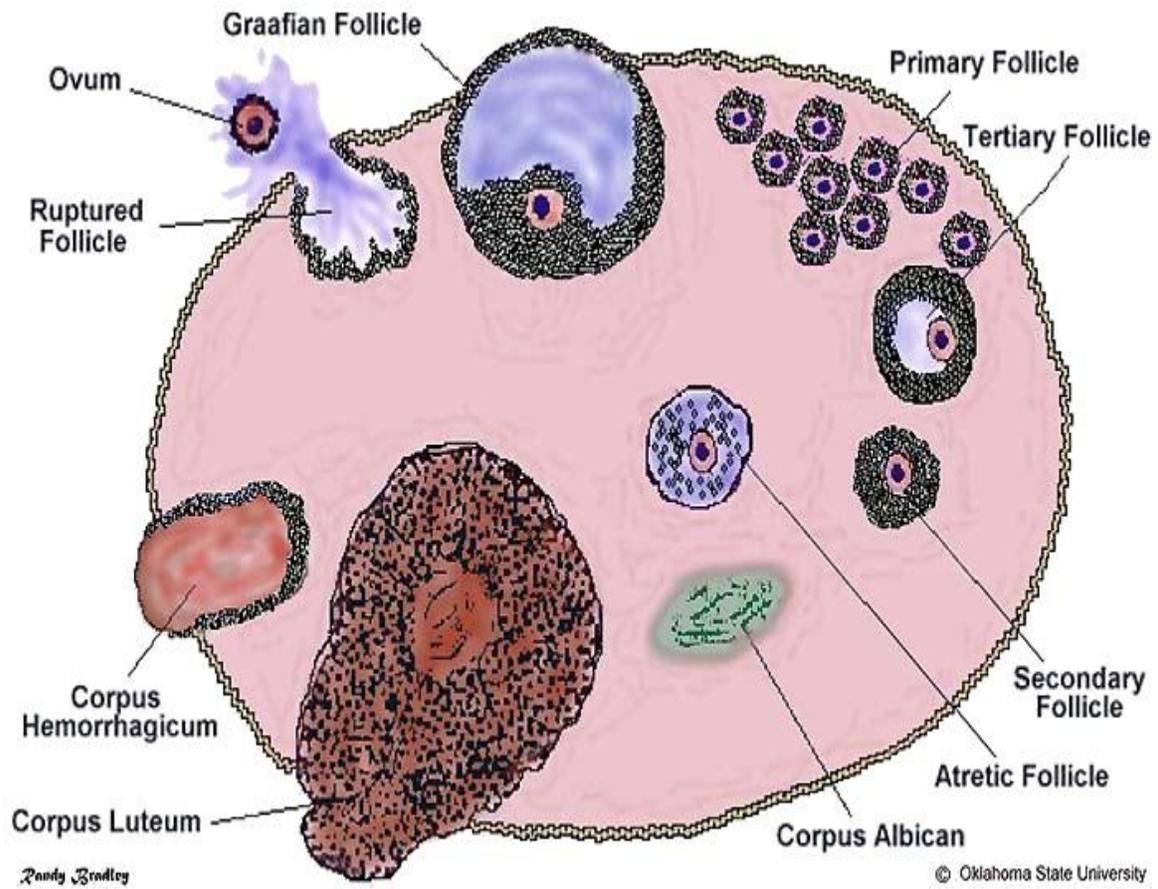
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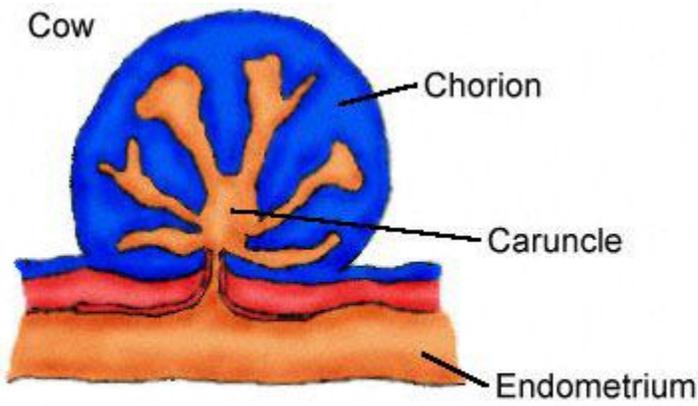
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## Bovine Reproductive Tract



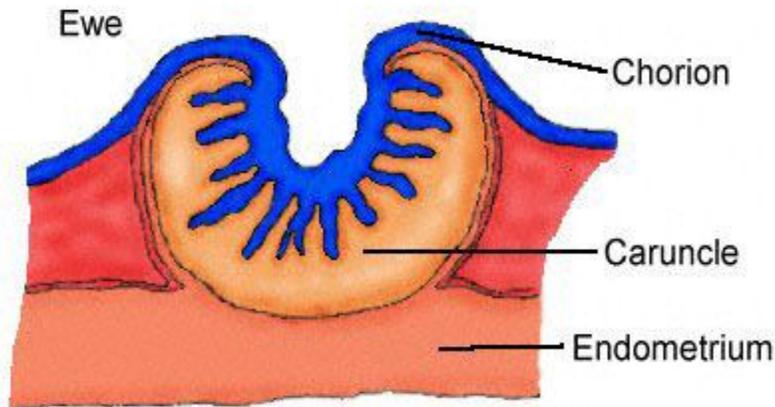






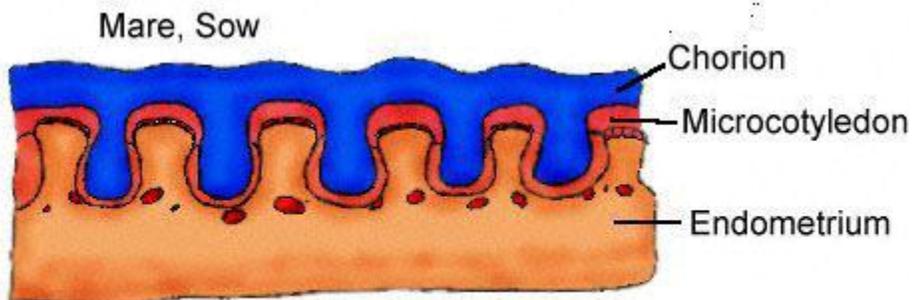
### Convex

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### Diffuse

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## COMPARATIVE ANATOMY OF THE FEMALE GENITALIA IN SMALL AND LARGE ANIMAL SPECIES

The female reproductive system can be divided into 3 major components:

- a. The female reproductive organ (gonads) i.e. the ovaries
- b. The reproductive tract - consisting of the Fallopian tubes, the uterus, cervix and vagina
- c. The external genitalia - consisting of the vestibule, clitoris, labia and certain glands which open into the vestibule.

### The Ovaries

The ovaries perform both exocrine and endocrine functions. They are the source of the ovum through the process of oogenesis and ovulation; and they are also concerned with the production of the ovarian hormones, i.e., estrogen, progesterone and relaxin.

The ovary is supported dorso-laterally by the broad ligament, and medially by the proper ligament. The shape, size and location of the ovaries vary with the species, the stage of the estrous cycle, and the stage of gestation.

The ovary of the cow is almond-shaped, but the shape is altered by growing follicles or corpora lutea. The average size is about 35 x 25 x 15mm which is larger in cows with active ovaries than those with inactive ovaries.

The ovaries of ewe and doe are almond-shaped and less than half the size of that of the cow.

The ovaries in the mare are kidney (or bean)-shaped and are two or three times larger than those for cows. The ovaries in the sow are slightly larger than those found in the ewe and appear as a “cluster of grapes”.

### The Fallopian Tubes (Oviducts / Uterine Tubes)

The paired Fallopian tubes are the means by which ova, released from the ovaries at ovulation, reach the uterus. Each tube is suspended in a peritoneal fold (the *mesosalpinx*) derived from the lateral layer of the broad ligament. The parts of the oviduct are the isthmus, ampulla, and, the infundibulum with its fimbriae.

The **isthmus** is the constricted portion lying next to the uterus; this merges with the dilated section known as the **ampulla** which makes up half the length of the oviduct.

The ampulla widens into a funnel shaped part called the **infundibulum**. The opening of the oviduct into the abdominal cavity is called the *ostium abdominale*, which is surrounded by a fringe of irregular processes called fimbriae, which form the cranial extremity of the tube. The isthmus or caudal end of the oviduct continues directly into the uterine horn at the uterotubal junction. In the mare, the isthmus opens into the uterine lumen through a small slit on a mound or papilla. In the cow and ewe, there is marked flexure at the transition of the isthmus with the elongated curving end of the uterine horn. The latter has a very narrow lumen. In the sow, the mucosa of the uterine tube projects into the uterine lumen as folds well supplied with blood. The length and degree of coiling of the oviducts vary in domestic animals. In the mare and cow, the oviducts are about 20 to 30 cm long and about 1.5 to 3.0 cm in diameter, while in the sow it is 15 to 18 cm in length. There is an intimate anatomical relationship between the ovary and oviduct. The fimbriated portion of the infundibulum has a partial attachment to the lateral side of the ovary and to the proper ligament of the ovary medially. At the time of ovulation, the infundibulum and its fimbriae assumes a very close proximity to the ovary and the ovum is swept into the fimbriated end by ciliary action and muscular contractions. The oviduct is the site of fertilization and early cleavage. Fertilization takes place in the lower portion of the ampulla following which the fertilized ovum or ova enter the uterus. The oviducts are tortuous, wiry and hard, feeling almost cartilaginous when rolled between the fingers. They are difficult to palpate rectally but manual vaginal and rectal examination, as well as palpation of the ovarian bursa are of assistance in their evaluation.

The infundibulum forms a bursa around the ovary in cat, rabbit, mink and others whereas it is separate from the ovary in the cow, doe, ewe, sow and mare.

### **The Uterus**

The uterus is a muscular-membranous structure designed for the reception of the fertilized ovum, for the nutrition and protection of the fetus, and for the initial stage of expulsion of the fetus at the time of parturition. In the domestic animals, the endometrium of the uterus is the only structure than can form sufficient placental attachment to result in normal development of the embryo and foetus.

The uterus consists of two uterine horns, a body and a cervix. The relative proportions of each, as well as the shape and arrangement of the horns vary from species to species.

In the sow, cow, doe and ewe the uterus is of the bicornuate type which is characterized by a small uterine body just anterior to the cervical canal and two long uterine horns. The horns are folded or convoluted and may be as long as four to five feet in the sow.

Fusion of the uterine horns of the cow and ewe near the uterine body gives the impression of a larger uterine body than actually exists and has sometimes resulted in their being classified as bipartite.

The mare has a bipartite uterus characterized by a prominent uterine body anterior to the cervical canal and two uterine horns that are not as long and distinct as in the bicornuate type. During pregnancy in the mare, foetal body extends into both horns, whereas the fetuses do not occupy the uterine body in the bicornuate type.

For the cow, sow and mare, the overall uterine length may range from 35 to 60cm. In the sow, doe, ewe and cow, the uterine horns account for 80 – 90 % of the total length while in the mare it accounts for about 50%. The uteri of the ewe and doe are less than half the size of the other species.

The duplex uterus which consists of two uterine horns each with a separate cervical canal which opens into the vagina is found in the rat, rabbit, guinea pig and other small animals.

The simple uterus, a pear-shaped body with no uterine horns is characteristic of human and other primates.

In late pregnancy, in the cow there are 70-120 cotyledonary attachments. There are 88-96 in ewes and does and these are smaller than those in cows. The sow and mare have a diffuse (surface) placental attachment. The extra-embryonic membranes lie in folds into the endometrium with chorionic villi extending into the endometrium in more fragile attachment than is found in the cow, doe or ewe. The placental attachment of the mare, sow, doe, ewe and cow is classified as **epitheliochorial**.

In humans and monkey, it is **haemochorial**. In dog and cat, it is classified as **endotheliochorial** whilst in rat, rabbit and guinea pig, it is classified as **haemoendothelial**.

### **The Cervix**

The cervix is the sphincter-like segment of the reproductive tract which anatomically and physiologically separates the uterine lumen from the vagina. It may be distinguished externally by its thick wall, due to the great thickness of the sphincter muscle, and internally by its constricted lumen.

The function of the cervix is to close the uterine lumen against macroscopic and microscopic intruders, and with few exceptions it remains closed at all times except during oestrus and at the time of parturition. At oestrus, the cervix serves as a passageway for sperm. In pregnancy, the cervical mucus hardens and seals off the canal by forming the so-called "**cervical plug**" or "**cervical seal**" which liquefies shortly before parturition. At parturition the cervix dilates allowing passage of the foetus and foetal membranes.

During natural mating in sow and mare, semen is deposited into the cervix.

The cervical canals in the cow, doe and ewe, have transverse interlocking ridges known as "annual rings" which help seal the uterus from contaminants.

The cervical canal in the sow is funnel-shaped, with ridges in the canal having a "cock-screw configuration" which conforms to that of the glans penis in the boar.

The cervical canal of the mare is more open than in other domestic livestock species, but mucosal folds in the canal which project into the vagina help prevent contamination.

### **The Vagina**

The vagina is the most caudal part of the internal reproductive tract. It is a muscular-membranous structure lying in the pelvic cavity dorsal to the bladder that acts as a copulatory organ and as a passageway for the fetus at the time of parturition. The vagina is capable of great dilation. Its caudal extremity is just cranial to the urethral opening in the region of the hymen.

The hymen is a slight circular constriction between the vagina and vestibule. Varying degrees of the hymen may persist in most species (with the exception of the bitch and queen), from a thin vertical central band to a completely imperforate structure. These usually disappear after copulation or parturition. Prominent hymenal folds are present in the mare and are referred to as transverse folds.

On the ventral floor of the vagina, beneath the mucosa and running the length of the vagina are the two Gartner's ducts which are remnants of the primitive mesonephric or Wolffian ducts. These ducts are present in the cow and are occasionally present in the mare, ewe and sow. Occasionally, cysts of these ducts are observed.

The length of the vagina varies with the species.

Cow: 26 to 30 cm in length in the non-pregnant animal;

Mare: 18 to 28 cm long and 10 to 13 cm in diameter when dilated;

Ewe: 7.5 to 10 cm long;

Sow: 7.5 to 11.5 cm long.

In the cow, doe and ewe, semen is deposited into the anterior ends of the vagina near the opening into the cervix during natural mating. The mucosal layer is of stratified squamous epithelium in most species with cow being the only exception.

The vestibule located between the vulva and vagina and comprised of the two labia, the dorsal and ventral commissures and the clitoris form the caudal termination of the genital tract. The urethra opens into the cranial ventral portion of the vestibule. The vestibule has several circular or sphincter-like muscles that close the genital canal to the outside. These muscles are attached to the perineal body, the sphincter muscles of the anus and the caudal and last sacral vertebrae.

Thus, during parturition the vestibule acts as the point of attachment for the entire genital tract to contract upon when expelling the fetus. The vulva lips normally come together evenly and do not gape, and the vulva opening usually lies at about 90 degree angle to the pelvic floor. The dorsal commissure is usually located about 4 cm above the ischial arch, while the ventral commissure is always pendulous and lies caudal and ventral to the ischiatic arch. The clitoris is about 3 to 6 cm long in the larger animals but is practically hidden in the tissues between the vulva and ischiatic arch. It is the homologue of the penis and in the mare and sow the glans clitoris is well developed and lies in a true fossa.

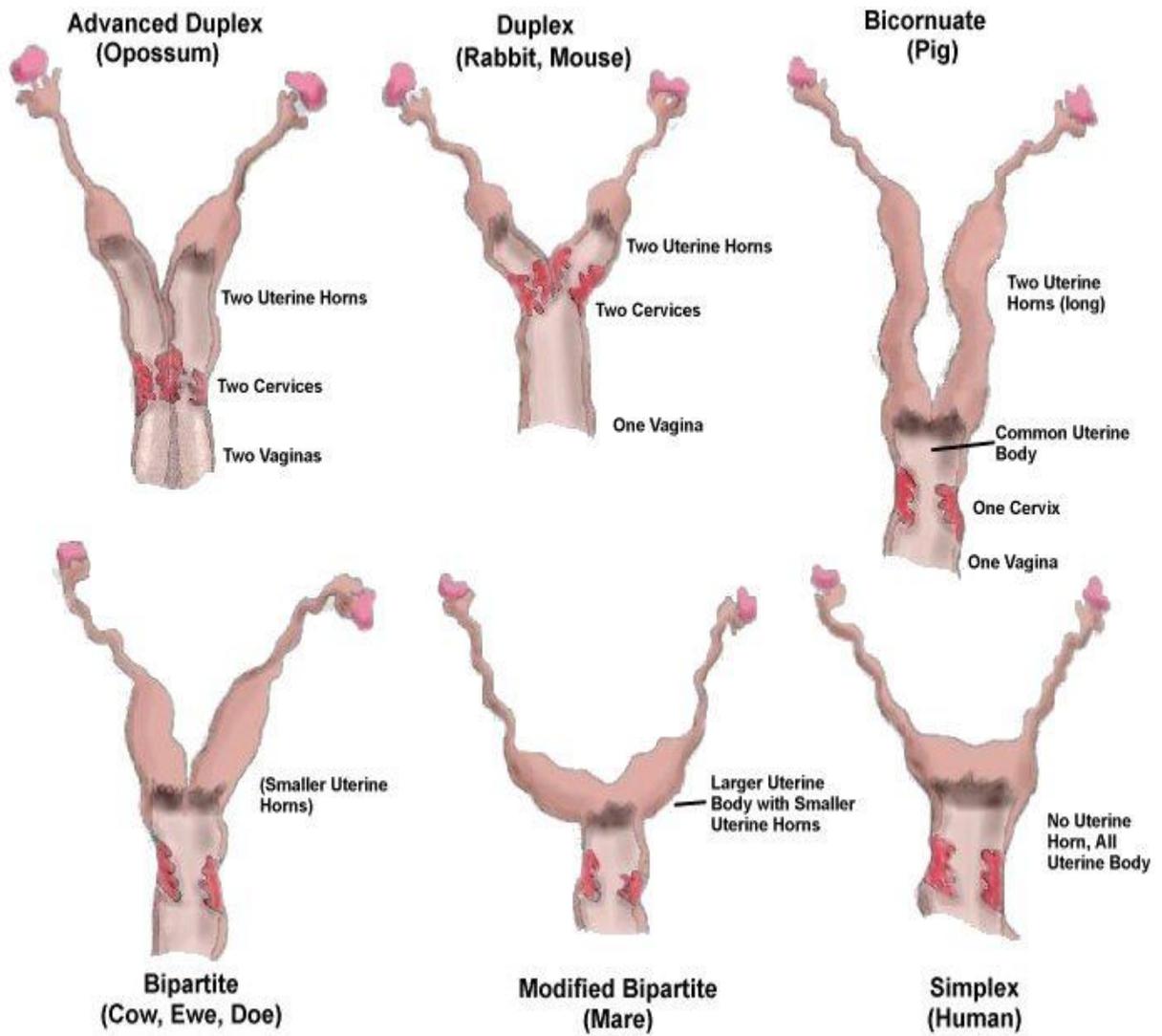
**Cow:** The vestibule of the cow is about 10 to 12.5 cm long on the ventral floor and 7.5 to 10 cm long on the dorsal wall. Beneath the urethral orifice is the suburethral diverticulum which is about 2.5 to 4 cm long. The external visible clitoris in the cow is small in size.

The major vestibular or Bartholin's glands are two in number and are located on either side in the constrictor muscles of the vestibule. These glands in the cow each open by a single duct in the lateral wall of the vestibule about 2.5 cm caudal to the vagina.

**Mare:**The vestibule of the mare is similar in size to the cow. Eight or ten ducts from the minor vestibular glands open through its dorso-lateral wall. In the mare the clitoris is large and prominent. The clitoral fossa and sinuses are homologous to the preputial folds and fossa glands of the stallion. The clitoral sinuses are 3 in number, with the central sinus being the largest, and located on the dorsal aspect of the glans clitoris. The narrow openings of the sinuses are generally hard to detect. Contractions of the vestibular and vulva sphincter muscles, elevates the clitoris and protrudes it between the vulva lips. This is called "**winking**".

**Ewe:**The vestibule and vulva of the ewe are similar to that of the cow, but the suburethral diverticulum is very small. Minor but not major vestibular glands are present. The clitoris is short and the glans concealed in a fossa.

**Sow:**The vestibule in the sow is fairly long, about 7 to 9 cm. The labia are thick. The minor vestibular glands are variable in number. The clitoris is located about 2 cm cranial to the ventral commissure. On either side of the cranial portion of the floor of the vestibule there is a cul-de-sac.



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## **FEMALE REPRODUCTIVE HORMONES**

Hormones are molecules that function as a message carrier within an organism, its only function is to convey information. Hormones are chemical agents which are carried by the blood to cells within a target organ of other target cells where they regulate a specific physiological activity.

### **ACTIONS OF GnRH AND ANTERIOR PITUITARY GONADOTROPHINS**

The hypothalamus secretes releasing hormones which influence the release of gonadotrophins from the anterior pituitary (adenohypophysis).

### **RELEASING HORMONES**

The following are the releasing hormones secreted by the Hypothalamus:

- i) Gonadotrophin releasing hormone (GnRH) - it functions in the release of Luteinizing hormone (LH) and Follicle stimulating hormone (FSH).
- ii) Thyrotrophic releasing hormone (TRH) – it causes the release of Thyroid stimulating hormone (TSH) and Prolactin.
- iii) Prolactin releasing factor (PRF) – it causes the release of Prolactin.
- iv) Prolactin inhibiting factor (PIF) – it inhibits Prolactin release.

### **ADENOHYPOPHYSEAL GONADOTROPHINS**

#### **i) FSH**

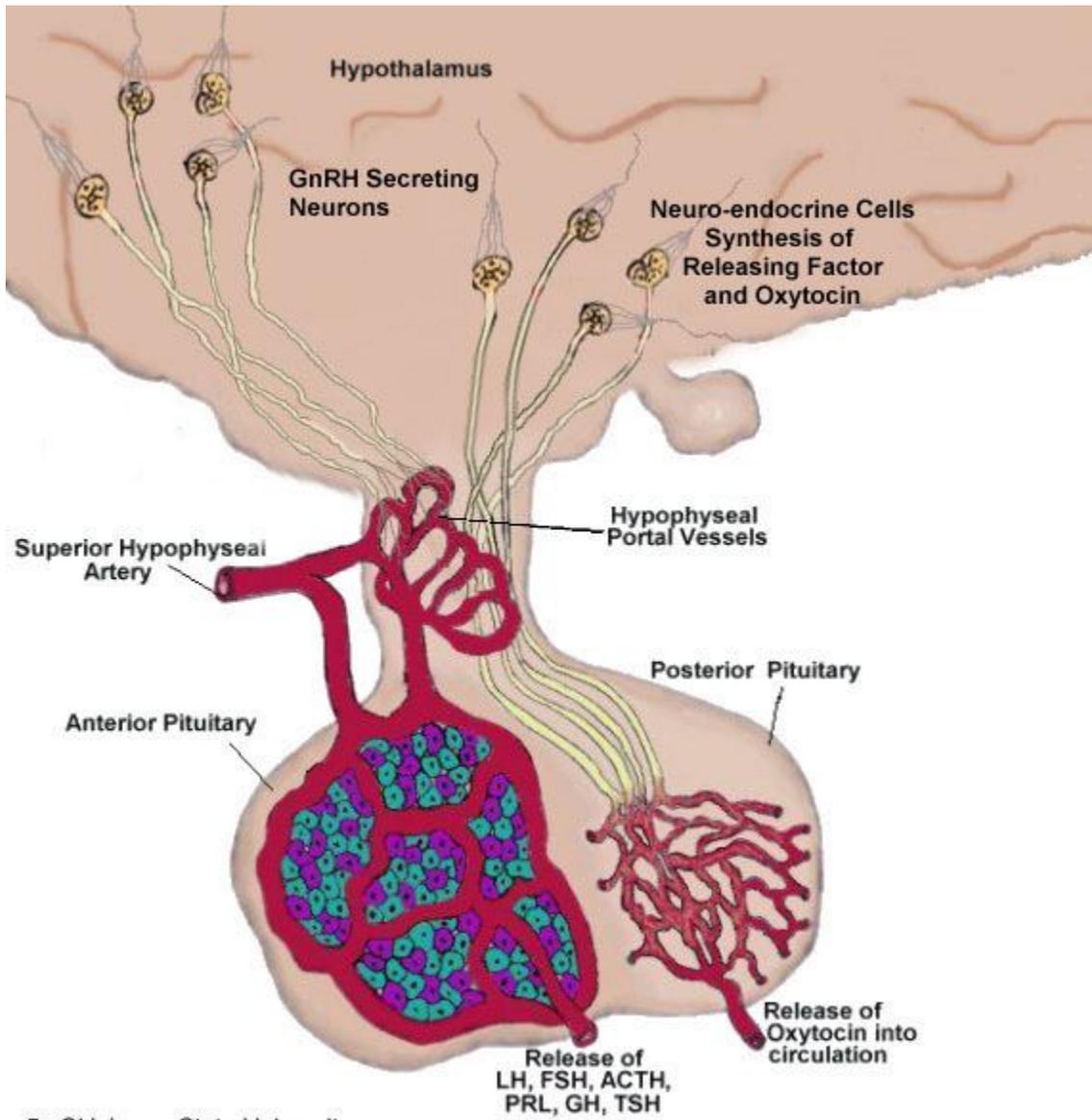
1. Stimulates follicle growth
2. Causes oestrogen production by granulosa cells in ovarian follicle.
3. Causes inhibin secretion by granulosa cells which is being enhanced by FSH and testosterone.

#### **ii) LH**

1. Causes production of testosterone from theca interna cells which is converted to oestrogen by granulosa cells FSH.
2. Causes oocyte maturation and ovulation.
3. Causes formation of corpus luteum (C.L) and subsequent production of progesterone.

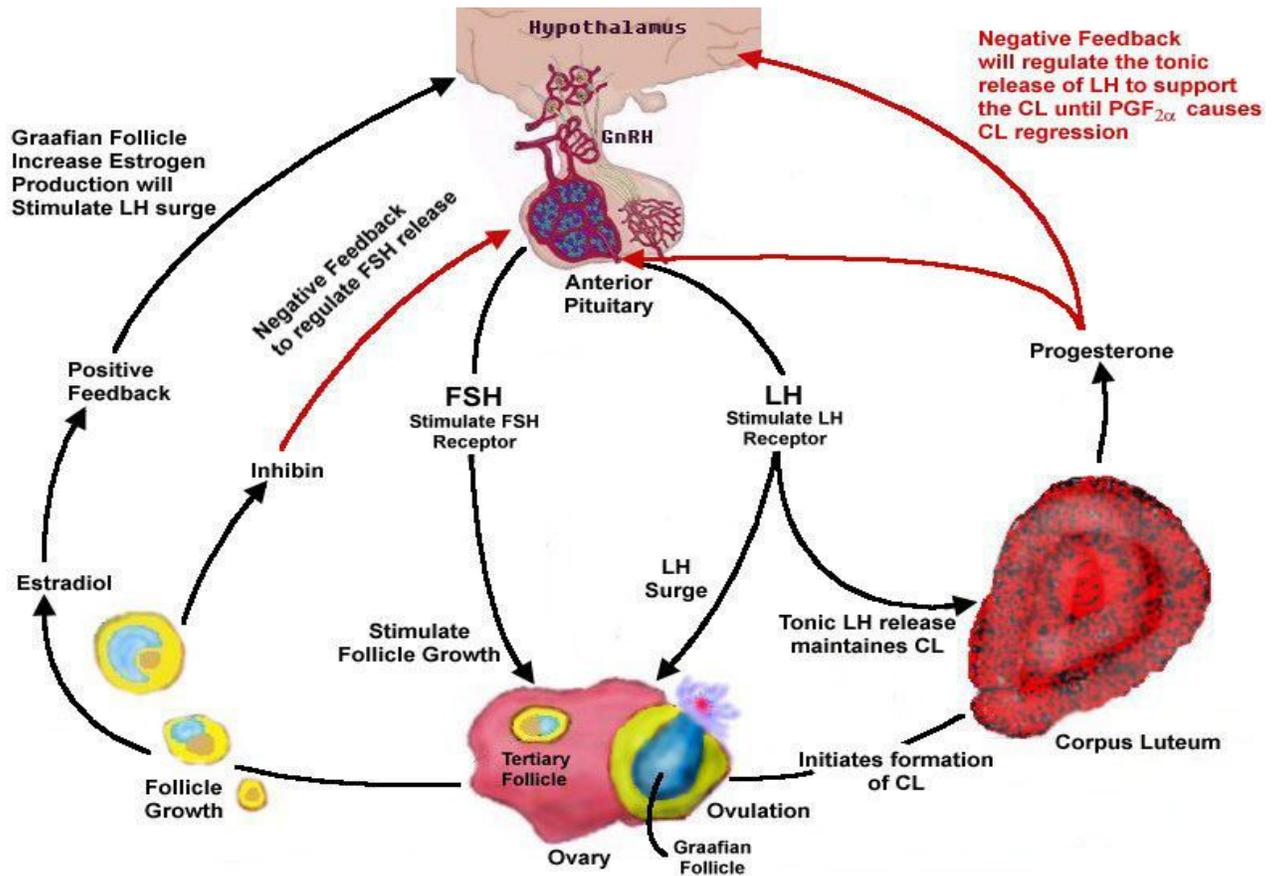
#### **iii) Prolactin**

1. Has synergistic effect with LH by LH receptor sites in CL to produce progesterone.
2. Causes development of the mammary gland.
3. Causes synthesis of milk.



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## Feedback Mechanism in the Female Animal



### NOTES:

- GnRH from the hypothalamus stimulates the release of FSH and LH from the adenohypophysis.
- FSH stimulates production of oestradiol and inhibin by granulosa cells in the ovarian follicles.
- Inhibin selectively prevents release of a FSH.
- When progesterone concentration is low, concentration of oestradiol stimulates a greater surge of GnRH, FSH and LH via a positive feedback control.
- LH stimulates production and release of progesterone by granulosa cells in the corpus luteum.
- High concentration of progesterone inhibit the release of GnRH, FSH and LH, this is a negative feedback control.

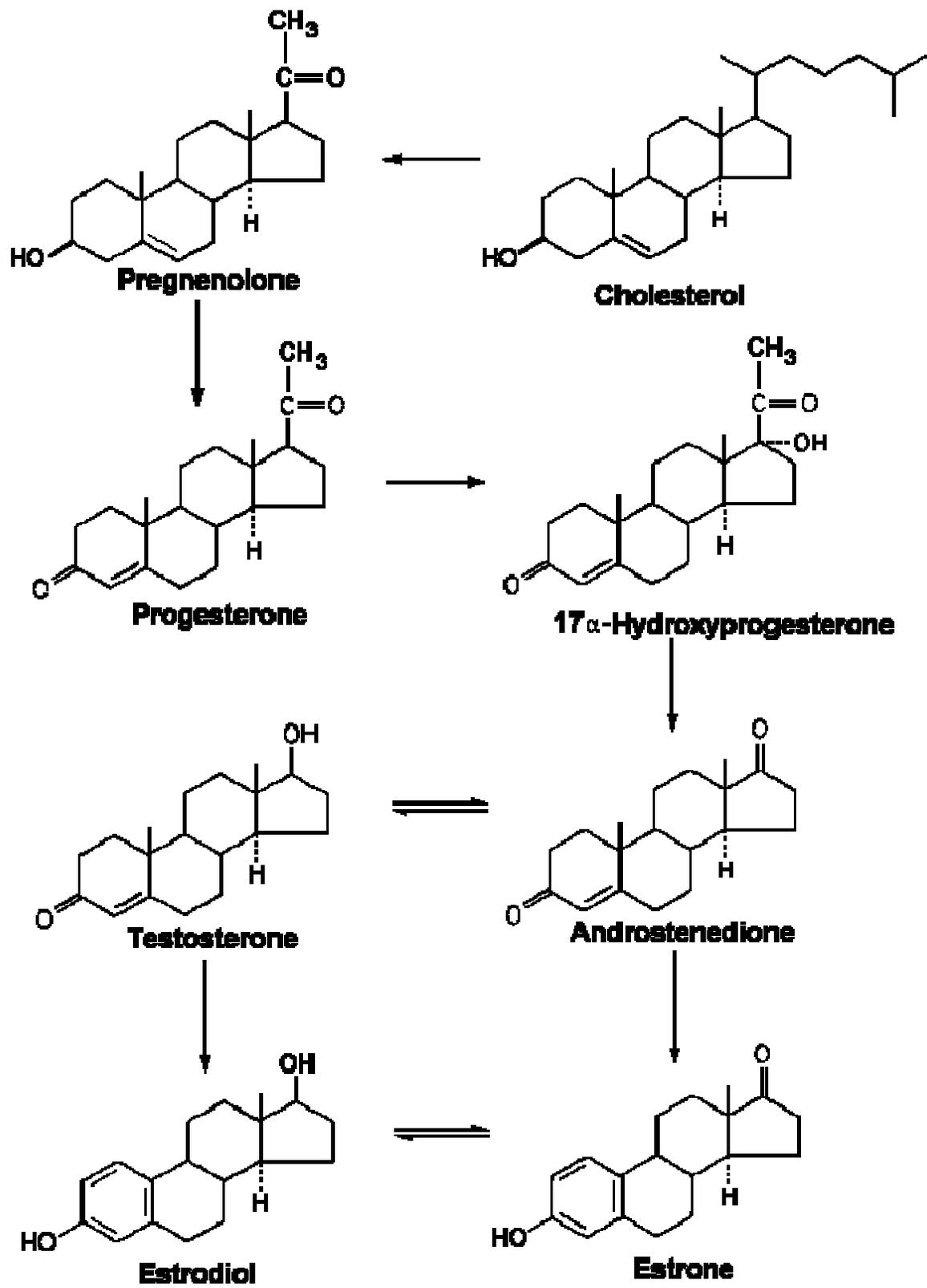
## STEROIDOGENESIS

### THE TWO CELL STEROID PRODUCTION THEORY

The two cell steroid production theory states that:

- Theca cells produce androgens under the influence of Luteinizing hormone while
- Granulosa cells convert the androgens to oestrogens under the influence of FSH.

	HORMONES	ROLES IN FEMALE REPRODUCTION
A 1.	STEROIDS OESTROGENS e.g. Oestradiol	i. Manifestation of mating behavior during oestrus. ii. Responsible for the cyclic changes in the female reproductive tract. iii. Enhances duct development in the mammary gland. iv. Promotes development of secondary sex characters
2.	PROGESTINS e.g. Progesterone	i. Inhibition of sexual behavior. ii. Maintenance of pregnancy via inhibition of uterine contractions and promotion of glandular development in the endometrium. iii. Promotion of alveolar development of the mammary gland. iv. Synergizes with oestrogen to prepare the uterus for pregnancy mammary gland for lactation. v. Regulates the release of GnRH via negative feedback control.
B. 1.	NON-STEROIDS OXYTOCIN – produced from the neurohypophysis	i. Stimulates the contraction of smooth muscle in the oviduct and uterus during parturition. ii. Elicits milk ejection reflex by stimulating the myoepithelial cells of the mammary gland. iii. It aids in sperm transport. iv. It helps in ovum transport
2.	PROSTAGLANDIN – found in the follicular fluid of Graafian follicles prior to ovulation e.g. PGF and PGE are the 2 most important for reproduction.	i. Causes regression of the corpus luteum. ii. Stimulates the smooth muscles of the genitalia.
3.	Inhibin – a protein hormone produced by the granulosa cells of the ovarian follicle.	It suppresses the release of FSH from the anterior pituitary.



## THE SOURCES, LOCATION AND ACTION OF MELATONIN, EQUINE CHORIONIC GONADOTROPHIC HORMONE (ECGH) IN FEMALE ANIMAL REPRODUCTION

S/NO.	HORMONES	SOURCE	ACTIONS
1.	Melatonin	Produced by the pineal gland. Its secretion is coupled to daylight i.e. decreased daylight stimulates its release	It is relevant in the seasonal breeders e.g. Ewe, Queen etc. It decreases reproductive activity in most species associated with decreased production of oestrogen and other reproductive hormones from the ovary and with an increased refractory time after mating before a subsequent mating occurs.
2.	Equine chorionic Gonadotrophic hormone (ECGH) = PMSG	Produced by uterus and placenta	i. Stimulation of follicular growth. ii. Stimulation of super ovulation in embryo transfer.

### PUBERTY IN FEMALE ANIMAL

It is defined as the achievement of the ability to reproduce or attainment of sexual maturity. It is the ability of an animal to release gametes and manifest complete sexual behaviour sequence. Irrespective of the mechanism, the attainment of sexual activity is a gradual process which is not a single event and occurs over a period of time.

The onset of sexual activity is associated with decreased sensitivity of the hypothalamo-hypophyseal axis to negative feedback control of the gonadal steroids.

In the prepubertal animal, the hypothalamo-hypophyseal axis is very sensitive to low levels of the gonadal steroids and these prevent the build up of the gonadotrophins which are necessary for ovarian cyclicity. This decrease in sensitivity to negative feedback of gonadal steroids i.e. allowing higher levels of gonadotrophins (FSH and LH) required for cyclicity results in the initiation of PUBERTY – this is termed “GONADO STAT” theory. The first heat of the pubertal animal is often silent.

Amongst females of the domestic species, puberty precedes the development of physical maturity and although they become capable of reproducing, their efficiency, particularly

with respect to their fecundity, has not reached its maximum. When the female animal reaches puberty, the genital organs increase in size. During the prepubertal period, the growth of the genital organs is very similar to that of other organ systems, but at puberty their growth rate is accelerated.

Females of domestic species reach the age of puberty at the following times:

Bitch: 6 – 20 months  
Cow: 7 – 18 months  
Dow: 4 – 8 months  
Ewe: 6 – 15 months  
Mare: 1 – 2 years  
Queen: 7 – 12 months  
Sow: 6 – 8 months

The changes that occur at puberty depend directly upon the activity of the ovaries. Generally age at first oestrus is not accompanied by ovulation in females thus it is not a true measurement of puberty. The age at first ovulation is difficult to be determined.

## **FACTORS AFFECTING THE TIME OF ONSET OF PUBERTY**

The onset of puberty is influenced by several factors, including age, genotype, season, body weight, nutrition and environment.

### **GENOTYPE**

The size of the female animal at puberty is determined genetically. More so, age at puberty is influenced by selection. Inbreeding delays puberty in heifers by retarding rate of growth. Breeds selected for milk and meat production reach puberty earlier than others not selected. Estimate of heritability for age at puberty range from 0.41 to 0.64. Small breeds reach puberty earlier as well as Crossbreeds (heterosis).

### **NUTRITON**

In most domestic species, the age of puberty is closely related to body weight which is a function of the nutritional status of the animal. And as such, animals that are well fed with good growth rates reach puberty before those that are poorly fed with slow growth rates. Poor nutrition delays puberty. Flushing may stimulate puberty via feeding of high energy feed.

### **SEASON OF THE YEAR**

In seasonal breeders, such as Ewe, Mare and Queen Cat, the age at which puberty occurs will be influenced by the effect of season of the year.

**PROXIMITY OF THE MALE (MALE FACTOR):** This is established in that exposure to the male of the species often advances the timing of the onset of puberty. This is probably being mediated by pheromones e.g. ram or boar effect.

## **VETERINARY GYNECOLOGY (VCT 502) LECTURE NOTES.**

### **REPRODUCTIVE CYCLE OF THE FEMALE ANIMAL.**

Theriogenology is the specialist branch of veterinary medicine that deals with *reproduction*, including the *anatomy*, *physiology* and *pathology* of male and female reproductive systems and the clinical practice of veterinary *obstetrics*, *gynecology*, *andrology* and *neonatology*.

What is reproduction?

Reproduction can be defined as sexual or asexual process by which organisms generate new individuals of the same kind.

Reproduction is the complex physiological process accompanied by or associated with the phenomenon which bears important relation to each other in approximately the following order.

- **1. Puberty**
- **2. Copulation**
- **3. Ovulation**
- **4. Fertilization**
- **5. Pregnancy**
- **6. Parturition**
- **7. Nutrition of the Newborn**
- **8. Involution of the Uterus**

Thus, while gynecology embraces no 1,3 &4(i.e. physiology and also pathology of female reproductive system).Obstetrics no 5,6,7&8 and andrology embraces no 2 as well as the physiology and pathology of male reproductive system including semenology and artificial insemination.

- Recall: Puberty is defined as the achievement of the ability to reproduce. In domestic animals, it is characterized by the expression of estrus in females and by the presence of sperm (*at least 50 millions of which >10% are motile*) in the ejaculates in males.

### **THE ESTROUS CYCLE**

- Following the onset of puberty in domestic animals there is the development of a physiologic rhythm of the reproductive system, called the estrous cycle.

- Estrous cycle can be defined as the physiologic, rhythmic, hormonal cycle of reproductive activities exhibited by sexually mature non pregnant female animals.
- In some animals such as the cow and sow estrous activity may occur throughout the year and these animals are said to be **polyestrous**.
- In mares, ewes, nannie, and queens estrous activity occurs during certain seasons of the year and these animals are referred to as being seasonally **polyestrous**.
- In bitches, their estrous activity occurs once or twice in a year depending on the breed thus referred to as **monoestrous/diestrous**.
- The estrous cycle of the domestic animals is divided into four phases or periods which blend one into the other - **proestrus, estrus, metestrus and diestrus**.
- Estrous cycle can also be divided into two periods based on the dominant secretory structure on the ovary
  - the estrogenic or follicular phase comprising proestrus and estrus
  - the progestational or luteal phase comprising of metestrus and diestrus.

**Proestrus:** The phase immediately preceding estrus. There is follicular growth and increased secretion of estrogen and regression of the corpus luteum of the previous cycle (**in polyestrous species**) and decreasing levels of progesterone.

**Estrus:** The period of acceptance of the male. The onset and end of this phase are the only accurately measurable points in the estrous cycle and hence are used as the baseline for determining cycle length.

Ovulation occurs during this phase in all domestic species with the exception of the cow (occurs 10-14 hrs after estrus).

Ovulation is a spontaneous process in all species with the exception of the cat, rabbit, ferret and the camel in which it is induced by the act of coitus.

**Metestrus:** The phase succeeding estrus. The granulosa cells of the ovulated follicle give rise to lutein cells which are responsible for the formation of the corpus luteum and increasing amounts of progesterone.

**Diestrus:** The period of the corpus luteum. The corpus luteum is fully functional during this phase and is producing large amounts of progesterone.

**Anestrus:** The prolonged period of sexual quiescence during which follicular development is minimal the corpus luteum although identifiable is regressed and is non-functional.

## ENDOCRINE CHANGES DURING ESTROUS CYCLE

The estrous cycle is controlled by the interaction of FSH, LH, estrogen and progesterone. These hormones are common to most domestic animals, however, their secretory patterns and relative effects vary among the species.

- During early development, prior to sexual maturity of the bitch, very little gonadotropic hormones are secreted and the ovaries, therefore, remain inactive.
- However, around the age of 6 months and above, the pituitary begins to secrete higher levels of the gonadotropic hormones called follicular stimulating hormone (FSH) and luteal hormone (LH). (Fig.1)

The rise in FSH and LH will initiate the sexual cycle and this cyclical increase and decreases in FSH and LH in turn, control

- the cyclic ovarian changes and, as such, are responsible for the physiologic events in the normal reproductive cycle of the female animals.
- The female animal has two ovaries that will produce the ova (eggs) which are contained within follicles that grow toward the surface of the ovary.

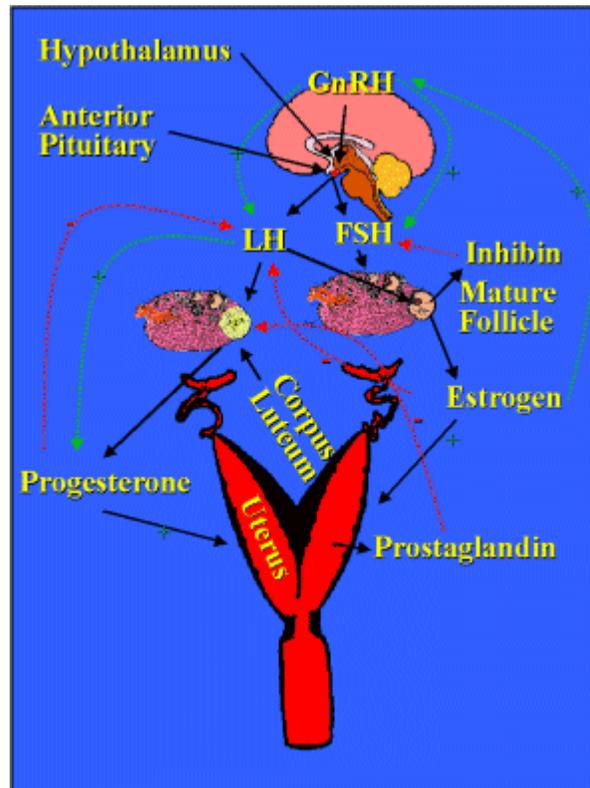


Fig.1 Illustrate the hormonal interaction during estrous cycle

- When FSH and LH from the pituitary gland begin to be secreted in high quantities during onset of sexual maturity, the ovaries and the follicles within them will begin to grow. (Fig.1)
- Within these follicles, a follicular fluid hormone, secreted by the ovary, called estrogen, surrounds the ovum. This hormone is a biologic chemical that produces physiologic and social/behavioral effects within the female that will signal a readiness to mate.
- Two days prior to ovulation, there is a surge in the secretion of LH by the pituitary gland preceded by rapid swelling of the follicle. This LH surge is of critical importance because in its absence, even with the other hormonal physiologic effects taking place, ovulation will not occur.
- Additionally, the LH surge causes the ovarian cells to switch over to secreting progesterone hormone rather than estrogen.
- Within two days of the LH surge, the follicle reaches the surface of the ovary and bursts, thereby releasing the ovum into a capsule that surrounds the ovary. This process is referred to as ovulation.
- In the meantime, the ruptured follicles from which each ovum was developed will begin to produce a rapidly dividing mass of cells called luteal bodies, which will make up the corpus luteum.(Fig.1)
- In addition to producing progesterone, which will maintain the pregnancy, the corpus luteum will also produce inhibin, the hormone that will signal the pituitary gland to decrease production of FSH and LH.

**COMPARATIVE REPRODUCTIVE PATTERNS AMONG FEMALE SPECIES**

<b>Species</b>	<b>Female</b>	<b>Estrous cycle length</b>	<b>Estrus length</b>	<b>Ovulation time</b>	<b>Estrus synchronization</b>	<b>First post-partum estrus</b>	<b>Seasonal breeders</b>
<b>Canine</b>	Bitch	varies	9dys	3-4dys of estrus	NA	Varies(4mths +)	No
<b>Feline</b>	Queen	varies	8-10dys	Induced by mating	HCG or GNRH to induce ovulation	2wks depending on nursing period.	Long day/polyestrou
<b>Bovine</b>	Cow	18-24dys	24hrs	18hrs after end of estrus/24-30hrs after LH peak.	11 day double injection of PGF or Progestagen for 14 days.	Varies(maximum of 83days post calving)	no
<b>Equine</b>	Mare	21dys	5-7dys	2dys before the end of estrus	PGFor Regumate for 14 days.	5-15days foal heat then 30dys after	Long day/polyestrou
<b>Ovine</b>	Ewe	16dys	24-36hrs	24-30hrs after onset of estrus	PGF or Progestagen for 10-14 variedays	Non seasonal 21dys infertile until 40dys.	Short day/polyestrou
<b>Caprine</b>	Doe	21dys	24-36hrs	24-30hrs after onset of estrus	PGF or Progestagen for 10-14 days.	21days	Short day/polyestrou
<b>Porcine</b>	Sow	19-23dys	2-3dys	Day 2 of estrus	Breed and abort with PGF	3-10 days(infertile except after weaning)	No/polyestrou

## THE CANINE ESTROUS CYCLE

The normal reproductive cycle of the bitch is comprised of four stages: proestrus, estrus, diestrus, and anestrus.

### PROESTRUS PHASE:

- Also known as the follicular stage because during this phase, the ovarian follicles, each containing ova, increase in size.
- Average duration is 9 days with a range of 3-17 days.
- Swelling of the vulva, the external tissue of the vaginal opening and bloody discharge marks the beginning of the proestrus stage.
- Males are interested in the bitch but the bitch is not
- Increasing amounts of estrogen hormone, secreted by the ovarian follicles, cause the cells of the vaginal walls to take-on a distinctive shape, a process known as *cornification*.
- Thus, vaginal cytology comprises of
  - Parabasal cells
  - Superficial cells increase. Cornification increases approximately 10%/day until about 100% of the cells are cornified.
  - May or may not see RBCs
  - May see a bloody discharge, yet see no RBC's on the vaginal smear.
  - WBC, mostly PMNs may be present.
- With vaginoscopy, the vagina mucosa glistens with rounded edges.
- Both the level of estrogen and vaginal cornification are useful indicators of proestrus.

### ESTRUS PHASE:

- Average duration is 9 days with a range of 3-21 days.
- The male and female are both interested in each other.
- The bitch will 'flag' her tail, as if allowing access to the vulva.
- NOTE: The estrus behavior results from the estrogen that peaked during proestrus abruptly declining..
- Physiologically, estrus coincides with the predominant presence of cornified vaginal epithelial cells and an increase in serum progesterone levels to 2ng/ml.
- Thus vaginal cytology comprises of:
  - The smear is very cellular. Many more cells than in anestrus.
  - Greater than 90% of the cells are cornified. Most of the cells are usually anuclear cells.
  - The PMN's are gone because the hyperplastic vaginal wall is too thick for them to cross the mucosa.
  - RBC's are generally gone.
  - The background of the smear becomes very clean.

- The cells may slough off on sheets as the end of estrus approaches.
- With vaginoscopy, the vulva and vaginal epithelium appear to wrinkle because the decreased estrogen results in water loss of the cells.
- Ovulation usually occurs 2 days following this increase in progesterone and hence, monitoring the levels of progesterone is an excellent indicator for timing breeding.
  - LH peaks about 24-48 hours into estrus and is caused by the estrogen peak during estrus. The LH surge causes ovulation of the ovarian follicles.
  - LH has very short peak duration and must be assayed every day in order to detect the peak rise. An in-house test is available to detect the LH peak, but you must test daily to identify the surge.
  - Ovulation occurs 24-48 hours after LH surge (day 3-4 of estrus)
  - Ovulation takes about takes 24 hours for all oocytes to be ovulated. A '2N' oocyte is ovulated and the oocyte must undergo reduction division, which takes 2-3 days, before the oocyte is ready for fertilization. If it is not fertilized, the oocytes die 3-4 days post maturation (5-6 days post ovulation).
  - The fertile period of breeding is generally recommended as the 3rd to the 5th days of estrus, or every other day of estrus.

#### **DIESTRUS PHASE:**

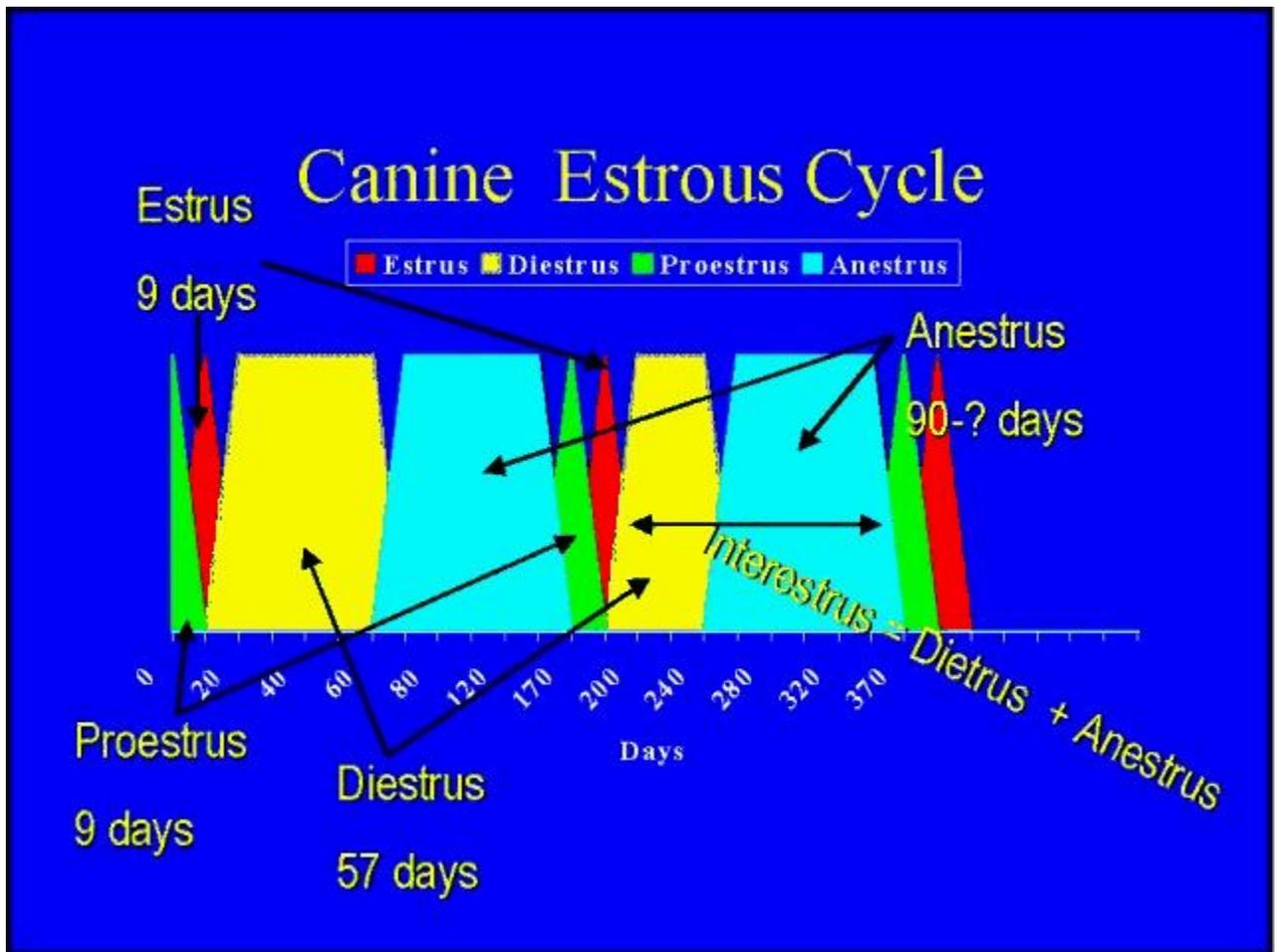
- Average duration is 2 months approximately 6 days after ovulation. The cornified vaginal epithelial cells will revert to a non-cornified state. This condition marks the beginning of diestrus.
- Thus the vaginal cytology comprises of
  - There is an abrupt change from the 100% to less than 50% cornification on the first day of diestrus.
  - The PMN's return to clean up all the sloughed cells and debris.
  - Intermediate cells as well as parabasal cells.
- Diestrus is the time of progesterone dominance. hormonal events of diestrus can be, for learning sake, identical in pregnant and non-pregnant bitches .
- Diestrus in the non-pregnant bitch could be called covert pseudopregnancy, since progesterone remains elevated, but there are no signs of pregnancy.
- The bitch refuses male's advances. This refusal is quite variable in when it occurs. .
- This stage ends when progesterone levels fall to less than 1 ng/ml just prior to whelping in the pregnant bitch or approximately 2 months after ovulation in the non pregnant bitch.

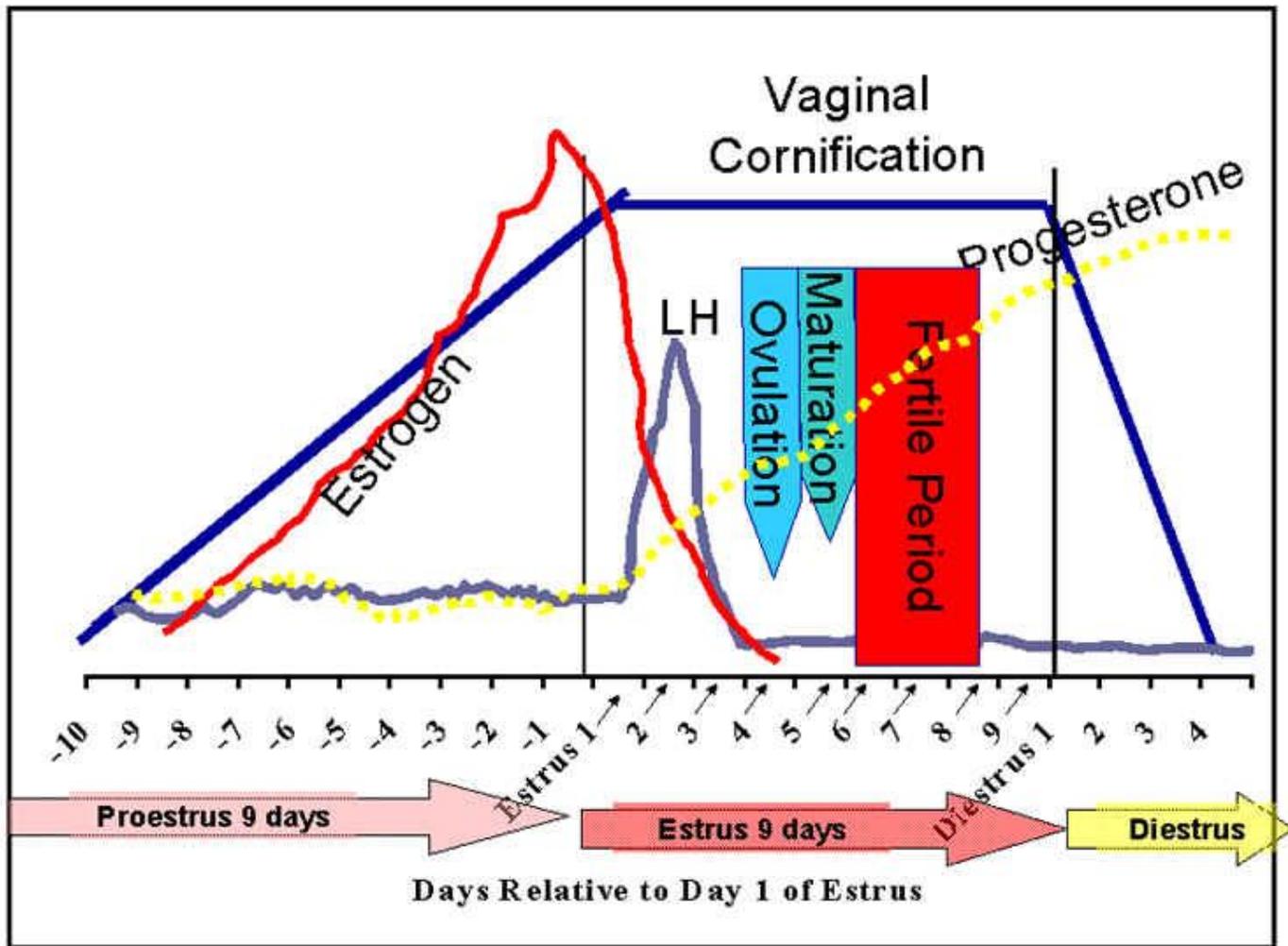
### ***ANESTRUS PHASE:***

- This is a time of mandatory endometrial repair.
- The endometrium is being 'repaired' after the progesterone effects during diestrus for the preceding 60 days.
- True anestrus lasts 90 -150 days post whelping, or post diestrus.
- Though Duration of anestrus is quite variable among bitches and may be governed by both genetic and environmental variables.
- Interestrous (anestrus + diestrus) lasts 150 - 210 days after the last estrus.
- Fertility is low if at least 90 day anestrus (or a 150 interestrous interval) is not attained. This is because the uterus has not repaired enough to maintain pregnancy.
- NOTE: Anestrus is not the same as interestrous. Anestrus is a variable time after diestrus while Interestrous is (diestrus + anestrus) averages 5 - 7 months.

#### Anestrus events:

- The male shows no sexual interest in the female.
- The female shows no sexual interest in the male.
- The vulva appears normal. It is not swollen or edematous.
- The vaginal cytology has very few cells and they are non cornified.
- The vaginal wall is very thin and appears pale on vaginal speculum examination.
- Progesterone is at baseline concentrations (<1 ng/ml). Even spayed bitches run basal levels of progesterone. This baseline progesterone is probably of adrenal origin.
- Prolactin secretion by the pituitary may promote anestrus, because prolactin inhibitors can be used to terminate anestrus (i.e. induce estrus).
- The beginning of proestrual bleeding marks the end of this stage.





## **THE FELINE ESTROUS CYCLE**

There are some peculiarities of feline estrous cycle which makes their reproductive pattern unique and distinct from that of other domestic species.

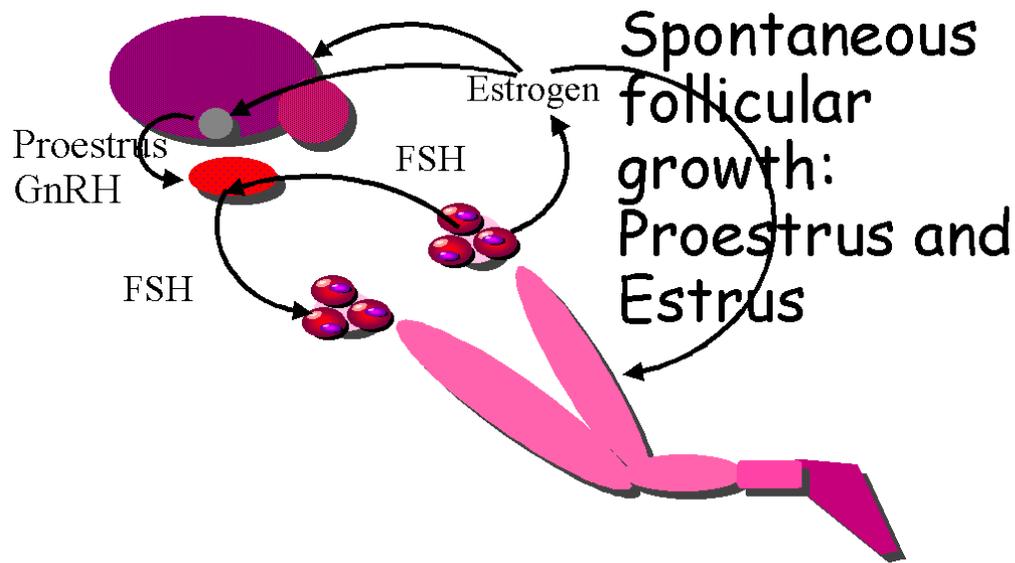
- Like the mare, the queen is seasonally polyestrous and responsive to photoperiod, thus referred to as long day breeders(i.e. requires 12hrs or more of light to maintain normal cyclicity)
- Although it has been noted that long haired breeds tends to be more seasonal than the short haired breeds.
- Like the bitch, the queen is unusual in maintaining sexual receptivity for a period of some days after ovulation, while the corpus luteum is forming.
- Most importantly from a clinical standpoint, like the rabbit and ferret the queen is an induced ovulator, requiring a copulatory stimulus or exogenous hormones for ovulation and corpus luteum formation.
- Puberty in the cat usually occurs at 9 to 10 months of age although may occur as early as 4 months or late as 2 years.
- However, because cats are seasonal breeders and the season in which the kitten was born influences the age at which puberty occurs.

The phases of the feline estrous cycle are identified as

- **Proestrus**
- **Estrus**
- **Interestrus**
- **Diestrus or pseudopregnancy**
- **Anestrus**

### **PROESTRUS PHASE:**

- Proestrus, the period preceding estrus, lasts 1 to 2 days. This phase is often unobserved and may be seen in only 16% of estrous cycles.
- During this time, the female is attractive to but do not accept the male.
- Behavioral changes may begin to be seen during proestrus in which-
- The queen may rub against objects, vocalize and assume a lordotic posture sometimes referred to as a “dragster posture”.
- She will place her front quarters on the ground, elevate her hind quarters and lift her tail to one side. When the dorsal caudal area is stroked, she will tread with her hind legs.



#### **ESTRUS PHASE:**

- Estrus is defined as the period of sexual receptivity. Estrus lasts 3 to 16 days (average of 7) and then subsides for 3 to 14 days (average of 9 days).
- Behavioral changes are more pronounced in estrus than in proestrus; however, no conspicuous changes in the appearance or size of the external genitalia are evident.
- The period following estrus is affected by ovulation whether induced by copulation or exogenous hormones.

#### **INTERESTRUS PHASE:**

- Interestrus is the period between successive estrus periods if ovulation does not occur.
- If the queen is not bred, she will cycle into estrus on an average of every 2 to 3 weeks.

## Estrous Cycle(s) of the Queen

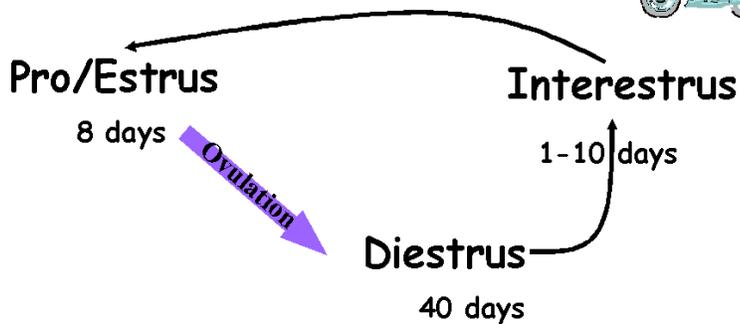


Repeats over and over  
if there is no ovulation

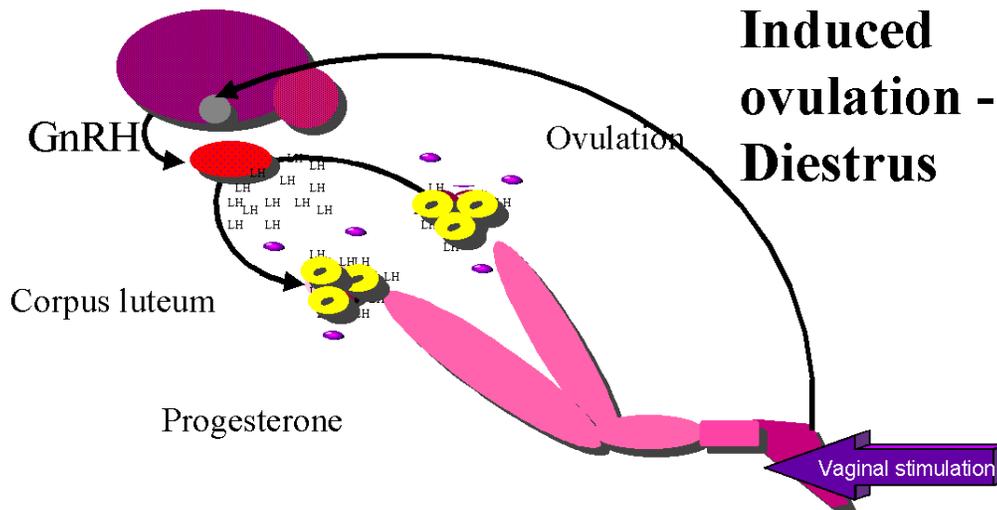
### DIESTRUS PHASE:

- If the queen ovulates, corpora lutea are formed and secrete progesterone. Note that elevated progesterone levels are the hallmark of diestrus.
- If the queen is not pregnant, diestrus is also termed *covert pseudopregnancy* and lasts for 35 to 40 days.

## Estrous Cycle(s) of the Queen



## OVULATION



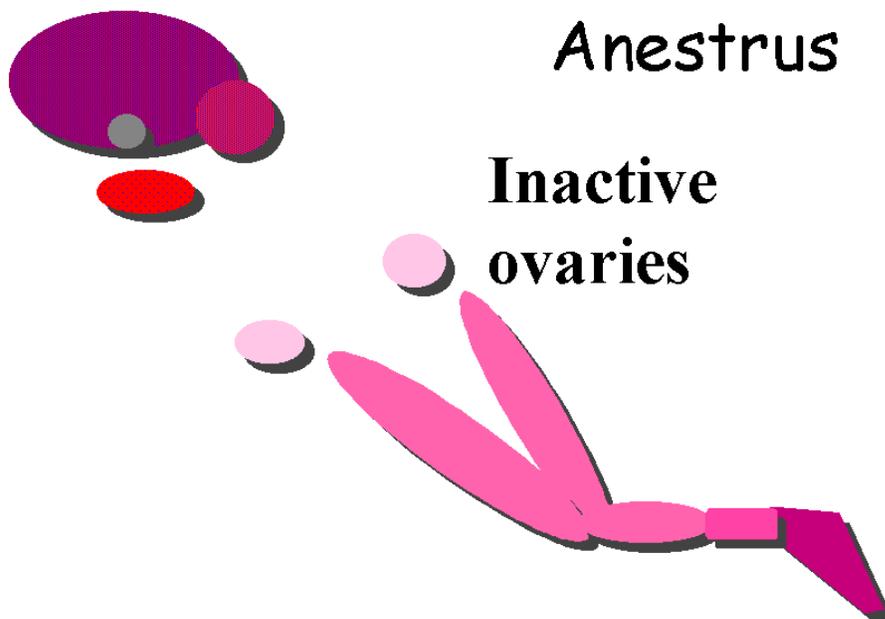
Queens are not spontaneous ovulators rather they are induced ovulators thus-

- Ovulation is induced by copulation (Ovulation reportedly occurs 24 to 60 hours post coitus and may vary depending on the mating pattern.)
- Ovulation is dependent on adequate LH release.
  - Both the peak concentration and the duration of elevation of LH are important in determining whether ovulation takes place.
  - Insufficient copulatory stimuli will fail to induce enough LH to be released to cause ovulation. LH release occurs within minutes of coitus and peaks approximately 1 to 2 hours later.
- The LH response to copulation, therefore, may vary depending on the day of estrus upon which coitus occur. If vaginal stimulation occurs before day 3 of estrus, the queen may not ovulate.
- Ovulation is also induced by mechanical stimulation of the vagina using a thermometer, glass rod, insemination pipette, swab stick or an ovulator.
- When using any of this you must give multiple stimulations at least 2hrs apart to induce sufficient LH peak.
- Stimulation of nerves in the vagina causes a reflex stimulation of the hypothalamus thereby releasing gonadotropin releasing hormone (GnRH) which acts on the anterior pituitary, resulting, in turn, in a release of luteinizing hormone (LH).
- LH, then, stimulates ovulation and the development of corpora lutea.

- Progesterone levels in the blood can be used to verify ovulation. Concentrations of progesterone greater than 1 ng/ml are considered indicative of ovulation. Peak levels of 35 ng/ml in the pregnant queen and 24 ng/ml in the pseudo pregnant queen are observed at approximately day 21 post coitus.
- Lastly, ovulation can also be induced using exogenous hormones.
- It's been reported that administration of 25ug of GNRH on the 2<sup>nd</sup> day of estrus resulted in all matured follicles ovulating in the treated queens.
- Also, HCG has LH like activity thus administering 500 i.u. of it on day 1 and 2 of estrus achieved maximal ovulation rates.
- Reports have verified however that spontaneous ovulation may occur in the queen.

### **ANESTRUS PHASE:**

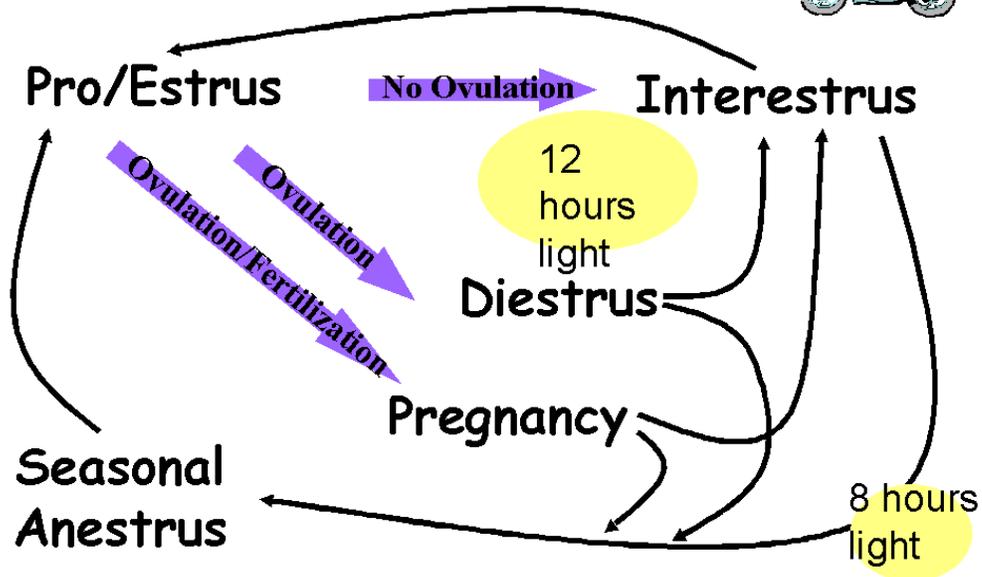
Anestrus is the seasonal period when the cat does not cycle.



### **Summarily note that:**

- Queens are induced ovulators, ovulating in response to vaginal/cervical stimulation from copulation.
- Following proestrus and estrus, there are three alternatives in the feline:
  - 1) ovulation does not occur, resulting in a return to estrus in 4 to 22 days (average 9 days);
  - 2) ovulation occurs without fertilization, resulting in pseudopregnancy;
  - 3) ovulation occurs and ova are fertilized, followed by pregnancy.
- Vaginal Cytology is not commonly performed in the queen because it may induce ovulation which may be difficult to interpret.
- Lastly queen's estrous cycle is diagrammatically summarized below.

# Estrous Cycle(s) of the Queen



## **ESTROUS CYCLE CONTROL IN BITCHES AND QUEENS**

The estrous cycle of dogs and cats are not as easily manipulated as in other species. Although estrous cycles could be controlled in various ways-

1. It could be suppressed/delayed/prevented.
2. It could be induced.

It should however be noted that return to normal cycling is highly variable and controlled studies are lacking with most protocols, and their use in valuable breeding individuals is not advised.

### **ESTRUS PREVENTION OR SUPPRESSION:**

- Permanent estrus prevention, of course, is best achieved by ovariectomy.
- No undesirable side effects, other than possible weight gain if fed ad libitum, have been associated with ovariectomy in bitches and queens.
- Ovariectomy is advisably performed on prepubertal females as young as 7 weeks of age.
  - At this age, the procedure is relatively simple and recovery is rapid compared to older animals.
  - No adverse signs have been seen when compared to performing gonadectomies at an older age.

For females that are to be used for breeding later, OVH is not advisable rather

- Progestagens can be used e.g. Ovaban which is Megesterol acetate, a synthetic progesterone.
- This can either be used to prevent the next cycle if administered (0.25mg/lb) at least 1 week before the next anticipated estrus cycle. The next heat will be in 4-5 months.
- Can be used to stop estrus when bitch is already in proestrus(1<sup>st</sup> 3 days), giving 1mg/lb/day for 8 days.
- NOTE: If the bitch should be bred during the first 3 days of treatment, consider mismatch management. If the bitch is bred after 3 days of treatment, pregnancy is unlikely to occur.
- This drug is 92 % effective at preventing the estrus if the bitch is in the first 3 days of proestrus.

However, the use of progestagens is not advisable in breeding females because it predisposes to Cystic endometrial hyperplasia-pyometra complex as well as other adverse effects (eg, mammary hyperplasia and neoplasia, hyperprolactinemia and lactation) though not as common in queens as in bitches.

- In queens, during the breeding season, begin treatment during Interestrus giving 2.5 mg daily up to 60 days will prevent a return to estrus.
- During the non breeding season, 2.5 mg given daily beginning during the interestrous interval for up to 60 days similarly delayed a return to estrus.
  - When suppression of estrus is desired, a dosage of 5 mg daily for 3 days or until signs of estrus ceases should be given. In this case, therapy is continued at a dose of 5 mg/week for 10 weeks or 2.5 mg/day for 60 days.
  - Other than weight gain, no serious side effects of megestrol acetate were observed.
  - More recently, significant adrenal cortical suppression has been reported with the use of megestrol acetate.
  - The queen should be allowed to have a normal estrus without hormonal manipulation before retreatment.
- Long term suppression of estrus in the bitch may be accomplished with a synthetic compounded androgen. Dose is 3µg / kg / day except for German Shepherds and their crosses, which require 6 µg / kg / day.
- Therapy must begin  $\geq 1$  mo before proestrus.
- It has been approved for a maximum use of 24 months, but it has been given as long as 43 months experimentally without causing problems.
- The next heat will be in 7 - 200 days (i.e. unpredictable) and will be of lowered fertility.
- If pregnancy does occur, any female pups will be masculinized.
- May cause clitoral hypertrophy, vaginitis especially in prepubertal bitches, increased activity of skin sebaceous glands, mild epiphora, and alterations in hepatic function.
- Fertility reported as normal on first estrus.
- **NOTE: It is not for breeding bitches**
- This synthetic androgen should not be given to cats because serious side effects preclude its use in cats.
- Possibly fatal hepatic disease; changes in thyroid function, serum cholesterol, thyroid histology and weight; and slight masculinization noted by clitoral hypertrophy and thickening of the cervical dermis.

### **INDUCTION OF ESTRUS:**

- Induction of estrus is possible in late anestrus bitches using prolactin inhibitors (e.g., Bromocriptine, Carbergoline).
- Estrus can be induced in the queen using follicle stimulating hormone or pregnant mare serum gonadotrophin (PMSG, eCG).
- The most successful protocol is administering 2.0 mg FSH IM daily until estrus, but for not more than 5 days.
- Using this protocol, normal fertility was observed in mated queens after estrus induction.

- Prolonged administration of FSH resulted in follicular ovarian cysts which subsequently failed to rupture.
- Queens experiencing an induced estrus may have an increased number of both unovulated follicles and corpora lutea than queens having a natural estrus.

## **DISORDERS OF REPRODUCTIVE CYCLE**

- Recall that during the normal reproductive cycle of the bitch, increase in the concentration of the hormone estrogen coincides with the proestrus stage.
- This elevation in estrogen results in cornification of the cells of the vaginal walls.
- The subsequent estrus stage (the acceptance period for mating) occurs when 90% of the vaginal cells are cornified.
- At the onset of estrus, estrogen levels begin to decrease while progesterone levels rise.
- Increase in progesterone levels coincide with the luteinizing hormone (LH) surge that will herald ovulation within the subsequent 48 hours.
- Throughout estrus, progesterone levels will continue to rise and estrogen levels will continue to fall.
- Loss of estrogen will cause the vaginal cells to revert back to a non-cornified state. When 50% or less of cells from a vaginal smear appears cornified, then the bitch is in the diestrus stage and the mating period has ended.

The mating cycle of the bitch is, therefore, controlled by strict regulation of hormones. As such, conditions that may interfere with normal hormone levels will interfere with successful reproduction and may result in the following disorders-

### ***1. PERSISTENT ESTRUS:***

- Persistent estrus is most often associated with a failure of estrogen levels to decrease during the estrus stage.
- Clinical symptoms of this condition present as prolonged (for 21 days or longer):
  - 1) cornification of the vaginal epithelial cells
  - 2) mating receptivity (willingness to "stand" for mating) and "tail flagging", and
  - 3) vulvar swelling.
- In prior years when exogenous estrogen treatment was used to terminate unwanted pregnancies, this condition was observed frequently while the bitch was receiving drug treatment.

- Since estrogen therapy is no longer advocated for pregnancy termination, occurrence of persistent estrus is now more often associated with endogenous (physiological) sources of estrogen.
- Such sources may include developing follicles (especially in bitches that may be treated with gonadotropin therapy to induce estrus), abnormal follicular cysts, or functional ovarian tumors.
- While less common sources include pituitary or hypothalamic tumors, or severe liver disease (porto-systemic shunt).
- Diagnosis of persistent estrus may be confirmed by cytological examination of vaginal smears, which will indicate persistent cornification of 90% or greater of the sampled cells.
- Monitoring serum estrogen concentrations is not a reliable method for diagnosing persistent estrus since many bitches cytologically diagnosed do not always demonstrate increased serum estrogen levels.
- Monitoring serum progesterone by ELISA may be more useful since a majority of bitches experiencing persistent estrus fail to demonstrate the normal increase (above 2 ng / ml) in progesterone levels.
- Ultrasonography is usually the first step to identify the source of endogenous estrogen. This non-invasive form of diagnostic imaging may be helpful for detecting ovarian cysts, follicles, or tumors.
- However, normal ovarian appearance on ultrasound does not rule out abnormalities.

Therefore, in the presence of confirmed persistent estrus where a source cannot be identified by ultrasound imaging, exploratory laparotomy with biopsy is accepted as the follow-up approach.

- In some instances, persistent estrus, particularly those cases associated with follicles or follicular cysts, will resolve spontaneously without treatment
- However, in those cases where the condition persists for longer than 3 weeks, treatment intervention is warranted.
- Treatment options for persistent estrus will be based on whether or not the owner has future expectations in regard to breeding the bitch.
- Ovariohysterectomy (spaying) is the preferred treatment for persistent estrus in bitches whose owners have no consideration of breeding. For those owner's who seek to salvage reproductive function within the bitch, alternative options may be considered.
- Injections of gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG), both of which induce the ovulation of ovarian follicles, have been successfully used in limited studies for the treatment of persistent estrus.
- Following administration of the treatment regimen, vaginal smears and serum progesterone levels are monitored weekly for indications of onset of diestrus.

- When treatment is successful, the cytological exam will show a decrease in the % of cornified cells and serum progesterone levels will increase within 2-3 weeks.
- Though ovulation may occur as a result of treatment, breeding is not recommended at this time.
- Some bitches that undergo successful therapy may subsequently develop a recurrence of persistent estrus: a condition which is highly indicative of a tumor. In such instances, ultrasound or laparotomy is indicated.
- Though progestin therapy with megestrol acetate (Ovaban®) is effective at reducing the symptoms associated with persistent estrus, progestin therapy should not be used in bitches that will later be bred.
- Progestin therapy results in a high incidence of cystic endometrial hyperplasia and pyometra, and therefore when this approach is used, ovariohysterectomy is considered mandatory within 3 weeks following treatment to prevent these secondary complications.
- As such, this is not a viable therapeutic option for owners wishing to preserve reproductive function.

## ***2. PERSISTENT PROESTRUS:***

- In this condition, estrogen levels fail to peak during proestrus.
- As a result, the estrus stage does not follow the proestrus stage.
- Though symptoms may appear similar to persistent estrus, examination of the vaginal epithelial cells shows only 50-90% of the cells in the smear to be cornified.
- Additionally, serum progesterone levels fail to reach 2 ng / ml.
- Treatment of persistent proestrus is the same as for persistent estrus.

## ***3. SPLIT ESTRUS:***

- Occasionally, a bitch will enter proestrus and fail to enter estrus or will experience a very short estrus.
- If the bitch is bred, conception will usually fail.
- However, the owner will note that within 3 to 4 weeks the bitch is demonstrating signs of entering proestrus again.
- Thereafter, the bitch proceeds normally through the subsequent stages of reproduction.
- This condition is known as Split Estrus.
- Split estrus occurs more commonly in young, first-estrus bitches, however, older bitches may also be affected.
- Split estrus usually resolves without the need for treatment.

- Recurrent incidences of split estrus in a bitch, however, may suggest chronic premature luteolysis (inability to maintain sufficient elevation of the serum progesterone concentration) or an underlying health disorder such as hypothyroidism.

#### ***4. RECURRENT ESTRUS (SHORTENED INTERESTRUS INTERVALS OR POLYESTRUS):***

- In some cases, bitches will experience only brief (shorter than 4 months) interludes between estrous cycles
- It has been observed that such bitches usually have a higher rate of infertility.
- The shortened intervals, however, are believed to be an effect rather than a cause of infertility.
- For example, it is believed that these bitches fail to ovulate, perhaps due to insufficient LH production, and as a result serum progesterone concentrations never get high enough to be recognized by the hypothalamus.
- This condition is believed to prompt the hypothalamus to initiate another estrous cycle.
- Fertile cycles may occur intermittently among infertile cycles, and as such, a bitch may conceive even after several unsuccessful attempts to breed during prior cycles.
- One study links recurrent estrus to functional follicular cysts and suggests that recurrent estrus, like persistent estrus, may respond to gonadotropin-releasing hormone (GnRH) therapy.
- Alternatively, mibolerone therapy (Cheque Drops) may be used to increase the interval between estrus cycles.
- When persistent estrus follows recurrent estrus, there is an increased suspicion for the presence of either an ovarian or hypothalamic/pituitary tumor.

#### ***5. PRIMARY AND SECONDARY ANESTRUS (PERSISTENT ANESTRUS):***

- Some reproductively intact bitches may completely fail to cycle.
- This may be due to numerous potential causes.
- The process to diagnose the reason for this abnormality can be quite extensive.
- Before undertaking this task, it is first essential to confirm the state of persistent anestrus
- Blood samples should be drawn on a monthly basis for 6-8 months for the purpose of measuring concentrations of serum progesterone. Progesterone levels in the normal reproductive bitch will rise above 2 ng/ml for 2 months after estrus.

- Failure to detect increased levels of serum progesterone over a 6-8 month period in the bitch will strongly suggest a state of persistent anestrus.
- Additionally, because functional ovaries will provide a negative control over hormones produced by the pituitary gland,
- Thus, other blood tests to detect elevated concentrations of luteinizing hormone (LH) or follicular stimulating hormone (FSH) may be indicative of abnormalities of ovarian development or premature ovarian failure that may bring about persistent anestrus.
- Once the condition of persistent anestrus is confirmed, then exploration to identify the underlying cause can be initiated..

## **CANINE BREEDING MANAGEMENT**

### **FACTORS TO CONSIDER BEFORE BREEDING-**

- Availability of good and comfortable homes for puppies.
- Behavioral problems – both the bitch and the male dog should be of sound temperament.
- Age of the pair (the bitch and the male dog should be sexually and physically matured.
- Hereditary Disorders – clearance from any hereditary disorders that are common to dogs breed (e.g. canine hip dysplasia) should be obtained before breeding.
- The pair's (the bitch and the male dog) medical and reproductive history. . They should also have up to date vaccinations and be free of diseases and physical abnormalities, including healthy reproductive tracts.
- The pair's body weight should be considered.
- Pelvic capacity of the bitch- this should be large enough to allow the passage of normal dimension fetuses.

### **ROUTINE PRE-BREEDING LABORATORY TESTS**

- Brucella canis screening testing (using the rapid slide agglutination test and agar gel immune diffusion test) should always be part of a pre-breeding workup in both male and female dogs especially in all outside bitches coming to a stud dog.
  - This may be very important because bitches are usually asymptomatic carriers of B. canis, and late/term abortion is often the first sign of infection.
  - Though it is unlikely that a male dog would be infected with B. canis without showing clinical signs, such as epididymo-orchitis.

- It is also very important to do a vaginal culture before an outside bitch is bred, however this may not be necessary if the bitch has no clinical signs or previous history of reproductive dysfunctions.

## **SCHEDULING THE BREEDING -**

Breeding management is an important component of successful planned reproduction in dogs. Mismanagement accounts for many of the breeding problems seen in practice. Because of the wide variation seen in normal canine reproductive cycles, it is not surprising that management issues could be confusing to both breeders and veterinarians alike.

There are some basic tools that are helpful in scheduling bitches for breeding that will result in successful mating and consequently pregnancy.

### **Methods used for timing a breeding include:**

- Behavior of the bitch, such as flagging the tail and standing to be mounted.
- Physical signs such as: vulvar swelling and bloody discharge.
- Vaginal cytology.
- Vaginoscopy.
- Hormone assay for either progesterone or LH which could be said to be best way to determine the ovulation timing which is the fertile period
- It is noteworthy to state that while vaginal cytology is fairly reliable; all other methods are unreliable except for hormone assay.

Thus the following behavioral, clinical, vaginal cytological and endocrinological changes will help to determine ideal time for breeding.

## **1. PROESTRUS:**

### **i. CLINICAL CHANGES:**

- The first evidence of onset of proestrus is characterized by vulval swelling.
- Grossly vulval labia become turgid, swollen, and warm to touch (coming into estrus).
- Simultaneously with this change or shortly thereafter (1-4 days) bloody vaginal discharge begins. Thus routine vaginal swabbing may detect bleeding earlier. This is useful in animals in which bleeding is light and difficult to see.
- Proestral bleeding is a result of diapedesis and subepithelial capillary rupture within the endometrium and cranial vaginal mucosa under estrogen stimulation.

- During proestrus, the uterus enlarges up to double its size, folds of the edematous cervix enlarge, and the squamous epithelium of the vagina and vestibule become hyperplastic. The vestibule become dilated, congested and edematous (vaginocopy).

ii. BEHAVIOR:

- Two weeks to a few days before onset of proestrus, the bitch may exhibit behavioral changes consisting of indifference, listlessness, and loss of appetite.
- During proestrus the bitch is frequently restless, excitable, does not obey orders, and may mount other bitches under kennel conditions.
- She may exhibit polydipsia and polyuria.

**The sexual behavioral changes are progressive:**

- In early proestrus the bitch attracts male dogs (due to pheromones in vaginal discharge in response to rise in estrogens); flirt, and allows anovaginal investigation by male dog, but *will not* allow mounting or coitus;
- She growls, moves away, snaps at male dogs attempting to mount.
- Towards late proestrus the bitch shows progressive interest in male dogs and vice versa she allows anovaginal investigation by other dogs; becomes calmer, more passive;
- Finally at estrus she stands to be mounted and permits coitus.

iii. VAGINAL CYTOLOGY:

- During proestrus the major cell types present in a vaginal smear are parabasal, intermediate and superficial epithelial cells, large numbers of red blood cells, and occasional white blood cells, *which soon disappear*.
- The smears also contain considerable amount of cellular debris.
- The earlier cytological indication of the onset of proestrus is the presence of erythrocytes in the smear.
- As proestrus advances towards estrus, there is progressive increase in the proportion of large polyhedral superficial cells, and decrease in amount of debris and RBC in the smear. Thus there are more mature cells and less parabasal and intermediate cells.

iv. ENDOCRINOLOGY:

- Pulses of FSH and LH secretion occur during late anestrus, which recruit cohorts of follicles for development.
- Estradiol concentrations increase from 5-10 pg/ml to peak concentrations of 50-120 pg/ml.

- Progesterone concentrations increase from 0.5 ng / ml to 1.5 ng / ml at the time of estradiol peak and then increase to 2-4 mg / ml at the time of LH peak, and 4-8 ng/ml at the time of ovulation.

## 2. ESTRUS:

Starts with period of first acceptance and ends at first refusal of male by the female dog. .

### i. CLINICAL CHANGES:

- Vulva is softer (relaxed, flabby), but slightly smaller than during proestrus.
- The vulval skin appears wrinkled, due to loss of turgidity.
- Vaginal discharge changes from bloody to straw colored in appearance and becomes less copious.
- On vaginoscopy, the vaginal mucosal folds are less prominent and less turgid.

### ii. BEHAVIOR:

- The transition from proestrus to estrus behavior includes interest and flirting to acceptance of the male.
- Note that upon stimulation of perineal region, dorsal lumbar areas and the vulva, the bitch in estrus will deflect the tail (flagging) and rotate her pelvis.
- She also responds to lumbar pressure by standing still and firmly, with the back arched (lordosis reflex).
- Vigorous massage of vulva and clitoris elicits thrusting response (test for estrus).
- Cessation of estrus behavior is usually gradual consisting of diminishing or intermittent receptive behavior.

### iii. VAGINAL CYTOLOGY:

- The vaginal cytological preparations during estrus contain largely or wholly large, multisided superficial nucleated or non-nucleated anuclear cells.
- Occasional erythrocytes and very little background debris.

### iv. ENDOCRINOLOGY

- LH surge occurs 24-48 hours prior to ovulation.
- Progesterone levels begin to increase before ovulation.
- The preovulatory increase in progesterone is necessary to elicit estrus behavior in the estrogen primed bitch.
- Also elicit vulval swelling and transport of ova after ovulation.

**ESTROUS VAGINAL CYTOLOGY PICTURE IS SUMMARIZED BELOW.**

<b>ESTR/STG RBC NEUT</b>	<b>PARAB</b>	<b>S/INTERM</b>	<b>L/ INTERM</b>	<b>SUPERF/ANUCL</b>	
<b>EARLY PROESTRUS</b> +	+++	+++	+/-	-	+
<b>LATE PROESTRUS</b> -		++	++	+++	+++
<b>EARLY ESTRUS</b> -	-	-	++	++++	+
<b>LATE ESTRUS</b> -	-	-	+	+++++	-
<b>DIESTRUS</b> +	++	+++	+	+/-	-
<b>ANESTRUS</b> +/-	+	+/-	-	-	-

**VAGINOSCOPY:**

- Vaginoscopy is also an accurate diagnostic breeding management tool in bitches.
- Vaginoscopy permits inspection of color and texture of the vaginal mucosa and of any abnormal structures that may be present and could be used with or in lieu of vaginal cytology.
- It may also help distinguish vaginal from uterine sources of vaginal discharge. A modified anoscope, proctoscope, or otoscope may be used.
- The scope must not be more than 8 to 15 mm in diameter and at least 10 to 20 cm in length with an adequate light source. .
- It should be inserted at the dorsal commissure of the vulva and directed craniodorsally until it is over the ischial arch, after which time the vagina proceeds forward in a more horizontal direction.
- In most bitches the cervix cannot be visualized through the vaginoscope because of the very cranial abdominal location of this organ, the nearly vertical orientation of the cervical canal, and the presence of the dorsal median post-cervical fold which obstructs the view of the cervix.
- However, under the influence of estrogen, the vaginal folds become swollen, moist, and pink.
- As a bitch progresses through proestrus and into estrus, these folds begin to lose their swollen appearance and become wrinkled.
- When the bitch is in full estrus, the vaginal folds have pronounced wrinkles with well defined edges).

- As the bitch progresses from estrus to diestrus, the vaginal folds become flattened, the vaginal mucosa takes on a red and white striped appearance.
- Vaginoscopy is easy to do and can be done in an awake, nontranquilized, standing bitch.

### **OVULATION TIMING USING HORMONAL ASSAY**

- It is noteworthy to state as earlier mentioned that ovulation is spontaneous in the bitch and generally occurs early in estrus (2-4 days).
- The average bitch will experience the LH surge on Day 10 (where Day 1 is defined as the first day that bloody discharge is observed), will ovulate on Day 12, and will, therefore, optimally conceive on Day 14.
- However, not all bitches ovulate on Day 12 following proestrus onset. Some may ovulate as early as Day 5 or as late as Day 25 in which case utilizing this standard ovulation schedule will result in breeding failure.
- Thus ovulation timing kits are used in canine breeding management.
- Ovulation timing kits are enzyme-linked immunosorbent assays (ELISA) that detect serum progesterone concentrations.
- During proestrus, a bitch is under the influence of estrogen but not progesterone. The first rise in progesterone concentration correlates with the ovulatory luteinizing hormone (LH) peak
- Thus ovulation timing kits allow indirect detection of the LH peak by detecting the first rise in progesterone concentration.
- It is recommended to test bitches every other day, usually starting on Day 5 of proestrus or when the vaginal cytologic examination reveals at least 50% superficial cornified epithelial cells.
- When breeding naturally or with fresh semen by artificial insemination breeding should take place the day that a progesterone concentration of 5 to 7.5 ng / ml is achieved and again 24 to 48 hours later.
- The same basic recommendation applies to cooled, shipped semen.
- When using frozen semen, intrauterine deposition of semen is best performed 72 hours after a progesterone concentration of 5 to 7.5 ng / ml is reached.
- This is the optimal time when the highest numbers of viable secondary oocytes are available for fertilization.
- Recently, an LH assay has been marketed (Status LH Luteinizing Hormone Test Synbiotics) for use in breeding management of bitches.
- The suggested use for this assay is to test blood samples daily starting on Day 5 of proestrus or after vaginal cytologic examination reveals at least 50% cornified epithelial cells.

- When using the LH assay, remember that a bitch might have numerous LH surges before the ovulatory LH peak. Always use a progesterone assay in conjunction with an LH assay to determine the optimal breeding time.

#### NOTE:

- Laparoscopic studies reveal that 75% and 95% of follicles ovulate within 72 hours and 96 hours respectively.
- Despite this apparent prolonged period of ovulation, corpora lutea are in comparable stages of development, suggesting that an endocrinological mechanism may synchronize ovum development so the puppies born will not differ in developmental age.
- It is the primary oocytes that are ovulated and undergo meiotic changes (maturation) in the oviduct which usually takes 2-4 days (average 60 hours).
- The secondary oocyte that is formed acquires fertilizable ability that is maintained for 1-2 days.
- Viability of oocyte is 4 days (3 days for primary and 1 day for secondary).
- Spermatozoa reach oviduct 25 seconds after natural mating; and large populations of spermatozoa are present in oviducts by 6-12 hours after mating.
- Reported intervals of sperm survival vary from 5 to 11 days and they maintain fertilizable capacity 3-6 days. Sperm capacitation time in the dog is approximately 7 hours.

#### FACTORS TO CONSIDER DURING MATING

- **Place of mating-** taking the female to male's established territory normally encourage a degree of aggression which is necessary for coitus to occur.
- **Frequency of mating** - it's been noted that conception rate following a single mating may be approximately 60 percent rising to greater than 80 percent with two matings.
- **Mating behavior-** if truly the bitch is in estrus she will stand to be mated after a period of courtship.

It is generally recommended that mating should be carried out early in estrus for maximum fertility, and there should be one mating every 1-3 days during estrus to maximize conception rates.

Litter size and conception rate can be increased by repeated services. In practice, mating is carried out on the first day of estrus and thereafter on alternate days until refusal. It is also advisable that service or artificial insemination should be

continued until the onset of diestrus to accommodate bitches with unusually long estrus.

### **QUEEN:**

- Proestrus is marked by an increase in the rubbing activity of the queen's head on inanimate or animate objects (including the owner and handler), often interpreted as affection.
- The behavior may continue for one or two days before the queen is sexually receptive. Sometime the proestrus may not be noticed.
- During estrus the queen will often vocalize and frequently assume a posturing position with the pelvis elevated, especially in the presence of a tom.
- While the queen is exhibiting lordosis, her tail is laterally deviated and the hind limbs tread actively.
- The male mounts by grasping the back of the queen's neck with its teeth than then begins a series of pelvic thrusts. Intromission and ejaculation occur after one-half to seven minutes from the time of mounting.
- The queen emits a loud cry, which is presumed to occur when the cervix and vaginal wall are stimulated by the 'spines' on the penis.
- This is followed by active rejection of the male by the queen. The queen cleans her external genitalia and rolls on the ground. After sometime (five to 20 minutes) the queen will posture again for another mating.
- If the pair is not disturbed they will mate at least five to seven times within two hours (30 times in 24 hours).

**N.B. A queen in estrus may not always mate for a few reasons (size incompatibility, unfamiliar surroundings, and personality).**

### **Breeding practices in queen:**

- The queen should be taken to the tom's territory. Multiple matings should be allowed a few times a day (3-5) until the end of estrus.
- Mature tom can be used three times a week or daily for 4-5 days in a row with a rest in between.
- For colony production, one tom for each 4-5 queens is the best ratio, and up to 15 queens / tom is the maximum.
- Overuse of tom may cause decreased vigor, decreased semen quality, and decreased conception rate.

## **CANINE MISMATING AND ITS MANAGEMENT**

Canine mismating simply means accidental mating resulting in a need for prevention or termination of pregnancy.

There are several conditions that usually necessitate/warrant termination of pregnancy in a bitch amongst which are:

- An accidental mating (mismating) of a young, first-estrus bitch.
- A valuable purebred breeding bitch that is accidentally bred by an "undesirable" dog,
- An estrous bitch that the owner may want to breed in the future that is found cavorting with a neighborhood dog in the backyard!
- An unanticipated health problem that suddenly develops shortly after breeding a bitch.
- An evidence that a bitch may develop life-threatening complications if allowed to deliver.
- In situations of chronic illness or where pregnancy in general may present a serious health risk to the bitch.

### **Regimens for the Management of Mismating**

Before discussing treatments for pregnancy termination, it should be noted that: just because a bitch in estrus is found together with a male doesn't mean they have already mated. In fact, it seems to be the case that a majority of bitches presented to veterinarians for "mismating" treatment are not pregnant.

One fairly reliable method of determining whether mating has indeed occurred is to examine a [vaginal smear](#) within a few hours, even up to a day, after the alleged liaison occurred.

If the dog was bred, one can almost always find sperm on the smear without much difficulty. However, not finding sperm does not rule out mating anyway, but suggests that it may well not have taken place, which can influence how the bitch is treated

**Ovariohysterectomy** (spaying) up to 3 to 4 weeks after mating is the first choice of many veterinarians to end an unwanted pregnancy and a finite way to ensure against the possibility of future pregnancies. However, in the case of mismated bitch for which a breeder intends to use at a later time in a breeding program, ovariohysterectomy is not a viable option.

Therefore, other non surgical methods are required to terminate the current unwanted litter while preserving the reproductive function of the bitch. Because hormones play an essential role in reproductive development, mating, and maintenance of a pregnancy, it is

not surprising that such non-surgical approaches to abort unwanted pregnancies focus on inhibiting or interfering with the intended function of these hormones.

### **NON-SURGICAL MANAGEMENT OF MISMATING:**

- There are three stages (trimesters) of pregnancy in the bitch. The first stage begins with fertilization and ends when the embryos implant in the uterus (approximately 20-22 days after the LH surge);
- The second stage begins at implantation and ends when the fetuses undergo ossification (40-42 days following the LH surge)
- The final stage begins at fetal ossification and ends at whelping.
- Ideally, if a pregnancy is to be terminated, the process should take place in the second stage.
- During the first stage, pregnancy diagnosis is uncertain and treating a possibly non-pregnant bitch to induce abortion exposes her to potentially unnecessary health risks associated with treatment.
- Abortion during the third stage of pregnancy will result in expulsion of the fetuses and because of the wide margin of variation in duration of pregnancy, the later the abortion the greater the risk for delivery of live puppies.
- Therefore, second stage abortions are the preferred time period because they will result in fetal resorption, which poses the least risk to the bitch, and offer less unpleasantness to the owner.
- No matter what method is employed, follow-up monitoring should be utilized to insure the successful completion of the procedure.
- For the monitoring, ultrasonography is the best method. Ultrasound imaging of the uterus should be performed 5-7 days following the treatment regimen.
- At such a time point, changes in fetal anatomy, placental detachment, fetal decomposition and resorption can usually be observed.
- If imaging indicates evidence of unsuccessful or incomplete termination, a repeat of the drug-treatment regimen is usually employed.
- Alternative methods for monitoring include utilizing tests that measure the decrease in serum progesterone levels associated with pregnancy termination.

#### **1. Progesterone-suppressing drugs:**

- Antiprogestin drugs, work by competitively binding to the progesterone receptor and thereby blocking the binding and biological effects of progesterone hormone, which is required to maintain pregnancy.
- Algepristone is one of the most successful and safe anti-progestin therapies used for preventing unwanted pregnancy in dogs
- It is extremely effective demonstrating a 94.8% to 100% efficacy rate at terminating pregnancy when administered to bitches from 0 to day 45 of pregnancy.

- Treatments with antiprogesterins in general are more effective and produce fewer or no side effects when used early in the course of pregnancy, prior to embryo implantation.
- Once embryos implant, treated bitches have a higher risk for developing symptoms of pseudopregnancy including mammary development and lactation.

## 2. Prostaglandins:

- Prostaglandins are natural inhibitors of progesterone that are synthesized late in normal pregnancy to reduce the amount of circulating progesterone and induce birth.
- Thus prostaglandins work by inhibiting the blood supply to the corpus luteum and promoting its degradation.
- Therefore, as prostaglandin levels rise, progesterone levels fall.
- Additionally, prostaglandin also induces smooth muscle contractions to promote expulsion of the fetuses from the uterus.
- To be effective, prostaglandin treatment must be administered at least 13-15 days following the LH surge or 30 days post mating when implantation has already occurred.
- Because many side-effects are obtained by using natural prostaglandins, synthetic analogues (such as Misoprostol) have been created to imitate the natural molecule while reducing the side-effects associated with use.
- Such side-effects can be severe and include excessive salivation, vomiting, diarrhea, loss of coordination, respiratory distress, anxiety and pupil dilation/constriction.
- However, even the analogues can cause significant complications so many veterinarians will combine prostaglandin treatment with parasympathetic agents such as atropine to reduce severity of the symptoms.
- Despite these approaches, bitches must still be hospitalized for the extent of the treatment regimen.
  
- **NOTE:** Women of childbearing age and people with asthma or other respiratory problems should use extreme caution in handling PGF solutions. This drug is readily absorbed through the skin and can cause uterine contractions and bronchospasm in exposed persons. This is another reason for conducting treatment of bitches in a clinic rather than by prescription.
- PGF analogs such as cloprostenol are not approved for termination of canine pregnancy. They are very much more potent than PGF, and using an analog at the same dosage as PGF<sub>2α</sub> can be lethal.

## 3. Dopamine Agonist Agents:

- Prolactin is the primary LH in dogs and is required for sustenance of the corpus luteum, which produces progesterone.

- Prolactin synthesis by the pituitary gland is stimulated indirectly by serotonin because serotonin inhibits dopamine, which is a direct inhibitor of prolactin.
- Therefore, increased levels of serotonin will block dopamine's ability to inhibit prolactin and prolactin levels will increase.
- Around day 25 to 30 of pregnancy after the LH surge, prolactin levels significantly increase.
- When drugs that stimulate dopamine (dopamine agonists), such as bromocriptine or cabergoline, are administered to the pregnant bitch it suppresses prolactin secretion and can terminate pregnancy in dogs by suppressing progesterone secretion from the corpus luteum.
- Dopamine [agonists](#) are effective in terminating canine pregnancy only after about 25 days of gestation. Like PGF, they require repeated treatment and commonly induce vomiting and inappetence.
- The major limitation to use of these drugs is that they are less effective and require high dose administration when used prior to day 40 of the pregnancy.
- Because inducing abortion in the pregnant bitch is not recommended after day 40, high-dose use of dopamine agonists has the effect of producing greater side-effects including vomiting and inappetence.

#### 4. New and Controversial Approaches

- **Dexamethazone**, a corticosteroid, has been used to induce abortion in dogs when administered intramuscularly every 12 hours for 10 days beginning at day 35 or 40 of pregnancy or when given orally 2-3 times a day for 5 days followed by gradual reduction of dose over the following 3-5 days.
- The exact mechanism of dexamethasone efficacy for terminating pregnancy is not understood, however, it is believed that it may cause degeneration of the corpus luteum and thereby reduce the level of circulating progesterone.
- Side-effects related to this treatment include loss of appetite, increased thirst, and increased urination. Because the exact mode of action is still unknown and because no studies have explored long-term effects of dexamethasone treatment on future fertility, this treatment is not recommended until further information is obtained.
- **Epostane**, a steroid which interferes with progesterone secretion by inhibiting the synthesis of the precursor molecule that becomes progesterone.

#### 5. Treatments No Longer Advocated

- **Estrogens:** at one time, large doses of estrogen prior to implantation of the fertilized blastocysts were utilized as a method to prevent pregnancy after mating.
- Fertilization of the ova takes place in the oviduct, the tube leading from the ovary to the uterus.
- For approximately 5-10 days after fertilization, the blastocyst will continue developing outside the uterus in the oviduct before the tubo-uterine junction opens allowing the blastocyst to finally migrate to the uterus and implant.

- When large doses of estrogen are administered to the bitch immediately after breeding, the tubo-uterine junction will remain closed and the blastocysts will degenerate in the oviduct.
- If given after day 10 of the pregnancy when the embryos have already migrated into the uterus, the estrogen will interfere with the development of the uterine glands, which will prevent the embryos from implanting.
- Side-effects resulting from use of high-dose estrogen are severe and potentially fatal including bone marrow toxicity with severe hematological side-effects often lead to death.
- Additionally, the abnormal ratio of estrogen: progesterone induced by high-dose estrogen treatment often leads to cystic hyperplasia of the uterine glands and pyometra, conditions that will preclude future fertility in the bitch.
- Though orally administered estrogen results in fewer and less severe adverse effects, clinical studies demonstrate that orally administered estrogen is ineffective at inducing pregnancy termination.

**NOTE:** Because effective termination of pregnancy is achieved only when utilizing high-doses of estrogen and because of the serious side-effects associated with this treatment, this method is no longer advocated as a means to end unwanted pregnancy in the bitch.

## **EQUINE GYNAECOLOGY NOTES**

### **Equine Oestrous Cycle and its Control**

The mare is a seasonal breeder, but is 'switched on' by increasing day length. The pineal gland is involved, since if it is removed the mare does not show a normal response to changes in photoperiod. In intact mares, melatonin concentrations increase during hours of darkness. The hypothalamus is responsible for the control of release of gonadotrophins from the anterior pituitary by the action of specific releasing and inhibitory substances which are secreted by the hypothalamic neurons, and are carried from the median eminence of the hypothalamus by the hypothalamic–hypophyseal portal system. GnRH stimulates the release of both FSH and LH in domestic species.

Specific neurotransmitter substances are involved in the regulation of the release of pituitary hormones. Noradrenaline stimulates the release of FSH and LH; the inhibition of the conversion of dopamine to noradrenaline blocks the 'oestradiol-induced' release of LH, which is responsible for ovulation; whilst serotonin inhibits the basal secretion of LH and regulates other neurosecretory systems. Dopamine also has an important role in the control of prolactin release. There is good evidence that in domestic species the secretion of FSH and LH is controlled by two functionally separate, but superimposable, systems. These are the episodic/tonic system, which is responsible for the continuous basal secretion of gonadotrophin and stimulates the growth of both germinal and endocrine components of the ovary, and the surge system, which controls the short-lived massive secretion of gonadotrophin, particularly LH, responsible for ovulation. There are two hypothalamic centres that are involved in controlling these two systems.

Not only does the anterior pituitary have a direct effect upon ovarian functions by stimulating folliculogenesis, follicular maturation, ovulation and corpus luteum formation, but the ovary has an effect upon the hypothalamus and anterior pituitary. This is mediated by oestradiol, produced by the maturing follicle, and by progesterone, produced by the corpus luteum. The episodic/tonic hypothalamic release centre is influenced by the negative-feedback effect of oestradiol and progesterone.

Inhibin and oestradiol act in concert in suppressing FSH secretion. Inhibin, which is produced by all antral follicles, has a longer half-life, and sets the overall level of negative feedback, whereas oestradiol, which is produced only by those antral follicles that have the potential for ovulation, is responsible for the day-to-day fluctuations. Two other peptide hormones have been isolated from ovarian follicular fluid; these have been designated activin, which stimulates, and follistatin, which suppresses, FSH secretion. The positive-feedback effect of oestradiol on hypothalamic-pituitary function is well demonstrated in farm animals, since the preovulatory surge of oestradiol stimulates the release of LH, which is so necessary for the process of ovulation and corpus luteum formation. The response of the anterior pituitary to GnRH is influenced by the levels of ovarian steroids so that there is increased responsiveness shortly after the level of progesterone declines and that of oestradiol rises. There are probably self-regulatory mechanisms controlling gonadotrophin secretion acting locally within the anterior pituitary and hypothalamus. Tonic release of gonadotrophins, especially LH, does not occur at a steady rate but in a pulsatile fashion in response to a similar release of GnRH from the hypothalamus.

The negative feedback of progesterone is mediated via a reduction in pulse frequency of gonadotrophin release, whereas oestradiol exerts its effect via reduced pulse amplitude. The onset of cyclical activity after parturition, at puberty or at the start of the breeding season is associated with increased pulse frequency of tonic gonadotrophin secretion. Progesterone is thus the main regulatory hormone which controls the oestrous cycle of the various species. Thus, when the concentration of progesterone in the circulation falls (associated with the regression of the corpus luteum) there is release of LH from the anterior pituitary.

The rise in LH triggers the secretion of oestradiol; this sudden rise stimulates the surge centre for the LH release and, as a result of this sudden increase, ovulation of the mature follicle occurs. Throughout the oestrous cycle, during pregnancy and other reproductive stages, there is dynamic follicular activity with growth and atresia; only about 1% of antral follicles subsequently ovulate. The CL is rapidly formed from the Graafian follicle after ovulation primarily from the granulosa and the thecal cells. The hormones which are most likely to be involved are prolactin and LH, perhaps in association with FSH.

In the normal, non-pregnant female, oestrus and ovulation occur at fairly regular intervals; the main control of this cyclical activity would appear to be the CL.

Luteal regression can be viewed from two aspects: Firstly, functional regression is rapid, so that the secretion of progesterone declines rapidly. Secondly, as regards structural regression when the CL is reduced in size, the latter process takes longer than the former. The sensitivity of the uterus to oxytocin is determined by the concentration of endometrial oxytocin receptors.

The CL becomes more sensitive to the luteolytic effect of PGF<sub>2α</sub> as it ages. The early CL is unresponsive to PGF<sub>2α</sub>. The manifestations of heat during the transitional phase are often atypical and make it difficult for the observer to be certain of the mare's reproductive status. Also, before the first ovulation, there is poor correlation between sexual behaviour and ovarian activity; it is common for the early heats to be unaccompanied by the presence of palpable follicles, and some long spring heats are anovulatory. However, once ovulation has occurred, regular cycles usually follow. The average length of the equine cycle is 20–23 days. Typically, oestrus lasts 6 days and dioestrus 15 days. Ovulation occurs on the penultimate or last day of heat. The mare raises the tail to one side and leans her hindquarters. The vulva is slightly oedematous, and there is a variable amount of mucoid discharge.

A mare which is not in oestrus will usually violently oppose the advances of a stallion, and for this reason when 'trying' mares at stud it should be done over a gate, box-door or stout fence. If the mare is in oestrus the stallion usually exhibits 'flehmen'.

### **Anomalies of the Oestrous Cycle**

The mare is notorious for variations or abnormalities in her reproductive cycle. This is in contrast to other farm livestock, which have been specifically bred over time for their ability to reproduce rather than to perform athletically. A wide variation in the length of oestrus is evident between mares, the extremes being between 1 and 50 days. In general, a variation can be seen with the time of year, longer and less distinct oestrous periods being evident during the beginning and end of the breeding season. Nutritional intake also causes variation in oestrous length.

When nutrition is limited, oestrus tends to be longer and less distinct, making it less likely that the mare will conceive during such a non-ideal time. This effect of poor nutrition may be an additional signal to the mare, indirectly indicating seasonal and therefore day-length changes.

The length of dioestrus also varies between mares, with the extremes being 10 days to several months. This delay is normally due to one of three reasons: first, a silent ovulation – ovulation occurred but it was not accompanied by oestrus, giving the impression that the mare has been in dioestrus for a prolonged period of time; second, the existence of a persistent CL – a CL that has not reacted to PGF<sub>2α</sub> or has not received enough PGF<sub>2α</sub> to elicit a response; or third, inactive ovaries, usually associated with the transition into or out of the non-seasonal state or true anoestrus. Other variations in the cycle do occur – for example, ovulation in dioestrus. LH is normally released in a low episodic fashion (1–4 ng/ml) during dioestrus; occasionally, however, these episodes are large enough to cause ovulation mid-cycle, despite the high dioestrus progesterone levels. This evidence of dioestrus rises in LH and the previously discussed second FSH peak mid-cycle indicates that, unlike many other species, progesterone does not serve to block completely gonadotrophin release in the mare. The converse, oestrus with no ovulation, has also been reported, normally in mares out of the breeding season. Covering at the foal heat is often unsuccessful, as fertility rates are normally low. Additionally the oestrous cycles following this foal heat have an increased chance of being disturbed, often showing prolonged oestrus and dioestrus, until steady cyclicity is achieved. The causes of many of these variations can be attributed to managerial or environmental influences, e.g.

nutrition, temperature, day length, etc. Occasionally, they are due to genetic faults, lactational effects or embryonic death.

### **Endocrine changes during the oestrous cycle**

The secretion of FSH is biphasic with surges at approximately 10–12 day intervals. One surge occurs just after ovulation, with a second surge in mid- to late dioestrus approximately 10 days before the next ovulation. It has been suggested that this increase in FSH secretion, which is unique to the mare, is responsible for priming the development of a new generation of follicles, one of which will ovulate at the next oestrus. The pattern of LH secretion is also unusual in this species since there is no sudden surge of this hormone but a gradual increase and persistence of elevated levels for 5–6 days on either side of ovulation. Oestrogens in the peripheral circulation reach peak values during oestrus whilst concentrations of progesterone and other progestogens follow closely the physical changes of the CL.

### **Peculiarities of Equine Seasonal Cyclicity**

The age at first oestrus, or puberty, is affected by nutrition and season of birth, and ranges from 7 to 18 months, with an average of 10 months before ovulation, the tension in the follicle usually subsides, and the palpable presence of a large fluctuating follicle is a sure sign of imminent ovulation. The onset of heat after foaling occurs on the fifth to 10th day. This foal heat is sometimes rather short, 2–4 days. It is traditional to cover a mare on the ninth day after foaling. The first two post-parturient cycles are a few days longer than subsequent ones.

During oestrus, a single egg is usually released, and there is a slight preponderance of ovulations from the left ovary. Twin ovulation commonly occurs in mares. However, there is a strong breed influence on twin ovulation; thoroughbreds are prone to it, but pony mares rarely show it.

All equine ovulations occur from the ovulation fossa; only at the ovarian hilus, but occasional protrusions of corpora lutea may be seen, but because of the curvature of the ovary and the presence of the adjacent substantial fimbriae these protrusions cannot be identified by rectal palpation. Just before the onset of heat, several follicles enlarge to a size of 1–3 cm. By the first day of oestrus one follicle is generally considerably larger than the rest, having a diameter of 2.5–3.5 cm.

During oestrus, this follicle matures and ruptures when it has attained a diameter of 3–7 cm. After ovulation, the other follicles regress, until, during the first 4–9 days of the ensuing dioestrus, no follicles larger than 1 cm are likely to be present. Several hours before ovulation, the ripe follicle becomes much less tense. The collapsed follicle is recognised by an indentation on the ovarian surface; there is usually some haemorrhage into the follicle, and the coagulum hardens within the next 24 hours. Quite frequently the mare shows evidence of discomfort when the ovary is palpated soon after ovulation. Unless sequential transrectal palpation or ultrasonic examinations are performed, it is sometimes possible to confuse a mature follicle with the early corpus haemorrhagicum, since before ovulation the follicular antrum is filled with follicular fluid and then soon after ovulation it becomes filled with blood. For this reason mares are sometimes incorrectly diagnosed as having failed to ovulate. For the next 3 days the luteinising mass

can be felt as a resilient focus, but later it tends to have the same texture as the remainder of the ovary.

In pony mares, however, of known history from daily examinations, it is possible to follow the growth of the CL by palpation because in ponies it forms a relatively large body in a small ovary. The CL attains maximum size at 4–5 days, but it does not protrude from the ovarian surface. On section of the ovary it is brown and later yellow and of a triangular or conical shape, with the narrower end impinging on the ovulation fossa. Its centre is commonly occupied by a variable amount of dark-brown fibrin. The cyclical CL begins to regress at about the 12th day of the cycle, when there is a parallel fall in the blood progesterone concentration. From this day onwards the events previously described recur. Ovulation, with the subsequent formation of a CL, does not always occur; the follicle may regress or sometimes undergo luteinisation. B-mode ultrasound imaging with a rectal transducer has been used to visualise follicles. This is particularly useful to predict the time of ovulation.

During winter anoestrus, both ovaries are typically small and bean-shaped. During the cycle, there are large variations in the ovarian size depending on the number and size of the follicles. During oestrus the ovary of the thoroughbred mare may contain two or even three follicles, each of 4–7 cm, and these, with other subsidiary follicles, combine to give it a huge size. During dioestrus, however, with an active CL and only atretic follicles the ovary may be little larger than in anoestrus. By visual examination of the vagina and the cervix using an illuminated speculum, it is possible to detect the preovulation period. In dioestrus, the cervix is small, constricted and firm; it and the vagina are pale pink, while mucus is scanty and sticky.

During oestrus, there is a gradual increase in the vascularity of the genital tract and relaxation of the cervix with dilatation of the os. As oestrus advances and ovulation time approaches, the cervix becomes very relaxed and its protrusion can be seen lying on the vaginal floor, with its folds oedematous; the vaginal walls are glistening with clear lubricant mucus. After ovulation there is a gradual reversion to the dioestrus appearance. During anoestrus, as in pregnancy, both the vagina and cervix are blanched; the cervix is constricted and generally turned away from the midline, the external os being filled with tenacious mucus.

On palpating the uterus per rectum, cyclic changes can be detected. With the development of the CL the uterus increases in tone and thickness, but these features diminish when the CL regresses. At oestrus, there is no increase of tone. During anoestrus and for the first few days after ovulation the uterus is flaccid.

During dioestrus, pregnancy and pseudopregnancy the cervix is identified on rectal palpation as a narrow firm tubular structure; at oestrus it is soft and broad. A temporary pneumovagina assists in this examination.

### **Signs of oestrus**

The mare becomes restless and irritable; she frequently adopts the micturition posture and voids urine with repeated exposure of the clitoris. When introduced to a stallion or teaser, these postures are accentuated; the mare raises the tail to one side and leans her hindquarters. The vulva is slightly oedematous, and there is a variable amount of mucoid discharge. A mare which is not in oestrus will usually violently oppose the advances of a

stallion. If the mare is in oestrus the stallion usually exhibits 'flehmen'. Good stud management requires that a mare is accustomed to the procedure and that, because of the interval between the end of the last oestrus and the start of the next, she is teased 15–16 days after the end of the last oestrus.

### **Mare Infertility**

On average 40–80% of services result in a live foal. Human interference in the reproduction of the horse has also had a significant effect on reproductive performance. Live foal rates for wild horses and ponies are in the region of 95%, compared with 60% for hand-bred mares. Embryo mortality or early embryonic death (EED) is held responsible for a significant amount of the apparent infertility. Rates of 5–14% have been reported for in-hand mating, compared with 1–2.5% for free mating, though higher rates of EED (up to 50% for normal mares) have been reported by others. As most EED occurs prior to day 40, most measurements of infertility will also include this. Reproductive performance has been largely ignored in the improvement of the equine. This is in contrast with other farm livestock, where reproductive efficiency is of utmost importance. It is evident that barrenness in a mare at the end of the season is not necessarily due to pathological infertility but is also a consequence of other environmental and managerial influences and the natural tendency in many mares to take a season off breeding. Some mares are consistently barren in early life but successfully breed later on. Failure to produce an offspring in a particular year is therefore a complicated problem and is not to be confused with infertility and EED.

**Fertile** able to produce a live foal

**Infertility** a temporary inability to reproduce

**Barrenness** lack of a pregnancy at the end of the season, but perfectly capable of producing a foal, as demonstrated in previous years

**Subfertility** inability to reproduce at full potential, may be temporary or permanent

**Sterility** a permanent inability to reproduce

**Embryo mortality** embryo loss prior to day or death (EED) 40, often occurring between scanning at day 15 and day 40

**Abortion** foetal death after day 40

**Stillborn** foetal death after day 300

**Fertilization rate** number of ova fertilized per ovulation (85–90%)

**Pregnancy rate** number of mares pregnant on a specified day, expressed per oestrous cycle (45–60%) or per breeding season (75–90%)

**Live-foal rate** number of mares foaling per number of mares bred over the season (50–85%)

In the mare, reproductive performance does not just depend upon successful gamete production, but also upon an appropriate environment for fertilization, free-living embryo, implantation, placentation and subsequent parturition. The failure of a mare to produce a foal at the end of a season may have numerous causes and, can be divided into **extrinsic** and **intrinsic** factors.

### **Extrinsic factors affecting reproductive performance in the mare**

Extrinsic factors affecting reproductive performance in the mare may be considered to include: lack of use; subfertile or infertile stallion; poor stallion management; poor mare management; and the artificially imposed breeding season.

#### *Lack of use*

A mare may not be covered in a particular season due to design or unavoidable circumstances. If she foals late in one season, she may not be re-covered, in order to allow her to return to foaling earlier in the year. Disease or infection may preclude a mare from use in a particular year, either because she herself is not in a fit condition to successfully carry a foal or there is a danger of systemic-or venereal-disease transfer. In the case of a performance horse, she may not be bred in a particular year due to work commitments, though advances in embryo transfer now potentially allow such mares to foal via use of a recipient mare.

#### *Subfertile / infertile stallion*

Half the responsibility for the success or failure of a covering lies with the stallion and half with the mare. If a mare is covered by an infertile or subfertile stallion, her chances of producing a foal are significantly reduced through no fault of her own. Mares can only be expected to perform to their full reproductive potential if they are covered by a stallion whose semen meets minimum requirements. A stallion must also be physically capable of covering a mare effectively; a good semen evaluation in the absence of the ability or willingness to cover is of no use in the natural service of a mare. To a certain extent, this problem may be overcome by the use of AI, but in such cases it must be certain that the stallion's lack of libido or ability is not due to a potentially heritable fault.

#### *Poor stallion management*

If any aspect of a stallion's management, especially his covering management, is not correct, then there is the potential for his fertility rates to be affected. Management in the earlier formative years also has a significant effect on a stallion's libido and hence reproductive performance. The imposition of an artificial breeding season causes problems with reproductive performance. Even with the use of artificial lights, a stallion's performance during the months of December to February is lower than during his true breeding season, so fertility rates for mares covered within this period of time cannot be expected to meet normal expectations. Behavioural abnormalities, often associated with inappropriate management, such as failure to obtain or maintain an erection, incomplete intromission and ejaculation failure, may cause apparently low fertilization rates, as can the incorrect detection of successful ejaculation.

#### *Poor mare management*

Any deficiencies or inadequacies in brood-mare management can lead to poor reproductive performance. Of specific significance is covering management, especially oestrus detection. Better, more experienced management and the use of veterinary diagnosis and hormonal manipulation of the cycle are seen to significantly improve reproductive performance. Stress associated with handling and transportation is suggested

to be associated with EED, possibly via changes in plasma cortisol and progesterone concentrations. Nutritional stress is also reported to affect reproductive success, again mainly via EED and abortion.

#### *Imposed breeding season*

There is currently considerable pressure for foals to be born as soon as possible after 1 January. Regardless of the treatment used, pregnancy rates out of the natural breeding season are never as high as normal expectations. As such, the continued imposition of an arbitrary breeding season places unfair constraints upon a mare's potential reproductive performance.

#### **Intrinsic factors affecting reproductive performance in the mare**

Intrinsic factors affecting reproductive performance in the mare may include age, chromosomal, hormonal, pituitary, ovarian, Fallopian tube, uterine, cervical, vaginal and vulval abnormalities and infections.

*Age:* It has the most significant bearing on reproductive performance. In general, fertility declines with age and EED increases. It is noted that young mares have 90–95% fertilization rates, with EED up to 50%. However, the fertilization rates of older mares decline to 85–90% and EED may reach 100%. Also, older mares yield more embryos with morphological abnormalities and demonstrate multiple ovulations and so an increased likelihood of multiple pregnancies thus further adding to the high apparent infertility rates in older mares. Despite reducing reproductive performance, provided a mare is in good physical condition, she may breed successfully well into her twenties. However, welfare considerations may preclude breeding a mare of advanced age. In such cases, embryo transfer may prove a viable alternative to putting an older mare through the stresses of carrying a pregnancy to term.

#### *Chromosomal abnormalities*

The normal chromosomal complement for the equine is 64 (32 pairs), the female complement being denoted as 64XX. Various variations on the normal complement include 63XO (a female with a single X chromosome), termed Turner's syndrome, one of the more common chromosomal abnormalities. Such individuals are characterized by small rudimentary ovaries, flaccid, poorly developed uterus, no ovarian activity and therefore permanent anoestrus. Such mares tend also to be short in stature. Mosaic chromosomal configuration may occur as a 64XX complement in some cells and 63XO in others; such individuals demonstrate erratic oestrous cycles with no ovulation. Very rarely, chromosomal complements of 64XX/65XXY are found; such horses are termed intersex. Positive diagnosis of chromosomal abnormalities is only possible by genetic mapping via cytogenic analysis of blood samples, though they may be indirectly indicated physiologically.

#### *Hormonal abnormalities*

The control of the mare's reproduction is a finely balanced cascade and interrelationship of hormones, involving the hypothalamic–pituitary–ovarian axis. Abnormalities / inefficiencies in any of these centres can cause imbalance throughout the whole axis. The

majority of hormonal deficiencies are associated with pituitary abnormalities – for example, Cushing’s syndrome. Complete failure, or neoplasia, of the pituitary is relatively rare in the horse, but temporary malfunction may occur, especially in association with the transitional period at the beginning or end of the breeding season. Mares during this transitional period tend to suffer from delayed oestrus, prolonged oestrus and dioestrus, silent ovulations, split oestrus (oestrus over a period of up to 3 weeks with possibly a quiescent period in the middle), etc. In such cases, it is evident that a period of time is required to allow the mare’s system to re-establish regular 21-day cycles. Diagnosis of hormonal abnormality is initially via a mare’s behaviour and the seeming inability to detect oestrus or, conversely, apparent continual oestrus. Diagnosis of the cause is helped via scanning and rectal palpation, by which ovarian activity can be monitored. The incidence of hormonal deficiencies or abnormalities is particularly evident today, as we continually attempt to breed mares earlier in the season. The use of exogenous hormonal treatments and / or light treatment does successfully advance ovulation and oestrus within the year, but does not eliminate the transition period, which may still be associated with problems.

Pituitary or hypothalamic tumours are rare in mares; they are associated with muscle wasting, hypoglycaemia, docility, alopecia, blindness and uncoordinated movement, in addition to prolonged anoestrus. Hormonal deficiencies during pregnancy may also result in reproductive failure. In particular, progesterone insufficiency may result in EED or abortion, depending upon when it occurs.

#### *Physical abnormalities*

##### **Ovarian Abnormalities**

Occasionally, ovaries may be absent, due to surgical intervention or chromosomal abnormality. Inactive ovaries and ovulation failure are often observed in mares and are exacerbated by the imposition of an arbitrary breeding season.

##### **Follicular atresia**

Follicular atresia is responsible for some incidences of ovulation failure. In such cases, a group of follicles will develop normally to about 3 cm in diameter, but there is a failure in the emergence of a dominant follicle, which would normally develop further. Conditions such as ovarian hypoplasia, granulosa cell tumours, ovarian cysts, uterine infections and malnutrition have all been implicated in follicular atresia. The best cure appears to be time, especially in mares encountering problems during the transitional stage of the breeding season. Often, succeeding cycles will not demonstrate the condition.

##### **Corpora lutea persistence and failure**

Corpora lutea (CL) persistence and conversely failure are also causes of reproductive failure in the mare, manifesting themselves as long or short oestrous cycles, respectively. Failure of the CL is less evident in the mare than persistence. However, CL failure is implicated in experiments using progesterone supplementation to prevent abortion. The presence of a persistent CL is more common in mares and is an important cause of anoestrus. The normal lifespan of a CL is 14 days, after which, in the absence of a pregnancy, the luteolytic hormone prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ), secreted by the uterine

endometrium, takes effect. A persistent CL is presumably, therefore, a result of failure in the release of  $\text{PGF}_{2\alpha}$ , or in the ability of the CL to react appropriately. The presence of such conditions in a mare is implicated by the lack of oestrous behaviour, and is confirmed by scanning or rectal palpation. The failure of the luteolytic message may be linked to uterine infection, rendering the uterine endometrium unable to produce  $\text{PGF}_{2\alpha}$ . EED and associated pseudopregnancy are other potential causes of a persistent CL. Treatment with exogenous  $\text{PGF}_{2\alpha}$  is normally successful.

### **Anovulatory follicles**

Anovulatory follicles (ovarian cysts or luteinized unruptured follicles) can be a cause of anoestrus. Most cysts are associated with follicles, though their presence must not be confused with large, perfectly normal follicles, which have been reported to reach up to 10 cm in diameter. Anovulatory follicles are characterized as large follicles that fail to rupture and ovulate. Instead, over time, they fill with blood and persist as haematomas, possibly over a number of cycles. Their presence is further complicated by their secretion of progesterone – hence their alternative name of luteinized unruptured follicles and their similarity to functional CL. Differentiation between the two can only be made at scanning by variations in their echogenic characteristics.

### **Ova fossa cysts**

Ova fossa cysts are reported, especially in older mares. They appear to be associated with the epithelium of the fimbriae and may cause blockage of the ova fossa and therefore a disruption of ova release and fertilization. They are often evident as a bundle of cysts, similar to a bunch of grapes, near the ova fossa. In the extreme, they may also interfere with the blood supply to the rectum.

### **Granulosa theca cell tumours**

Granulosa theca cell tumours are the most common tumours within the equine ovary and an important cause of anoestrus. They normally affect mares between the ages of 5 and 7 and are usually associated with a single ovary. The ovaries are either polycystic or large solid structures and may weigh up to 8 kg. The symptoms demonstrated by such mares depend on the hormones secreted by the tumours. Oestrogen, the most common, may result in nymphomaniac behaviour (prolonged oestrus) in the absence of ovulation. Testosterone-producing cysts result in stallion-like behaviour. Elevated inhibin concentrations are thought to cause the characteristically small contralateral ovary, due to negative-feedback effects. Removal of the affected ovary allows the resumption of normal reproductive activity by the remaining ovary.

### **Ovarian teratomas**

Ovarian teratomas, arising from germ cells and containing hair, teeth, bone, cysts, etc., have been reported but very rarely occur and, unlike teratomas seen in stallions, are benign. They are unilateral in occurrence, allowing the other ovary to function normally; pregnancy rates may or may not be affected significantly. Parovarian or paroophoron cysts are also reported, but rarely cause problems.

### **Hypoplasia**

Ovarian hypoplasia (underdevelopment) is a further cause of anoestrus. It is characterized by small, immature ovaries, with no ovarian activity or increase in ovarian size within the breeding season. Hypoplasia is usually bilateral and is often associated with chromosomal or hormonal abnormalities.

### **Cystic ovaries**

The term cystic ovary implies the presence of fluid filled structures within the ovarian stroma, which are hormonally active. Such structures are reported in cattle but are reported not to occur in mares.

### **Other abnormalities**

Dysgerminomas (malignant tumours of germ-cell origin), abscesses and haematomas (overflowing of the follicular cavity with blood after ovulation) are also reported to occur, but rarely.

### **Multiple ovulations**

Multiple ovulations, and the resultant multiple pregnancies, are major cause of EED and abortion and hence reproductive failure. The mare's tract is not competent to satisfactorily maintain more than one foetus. Twins are therefore not desirable and management in general is geared towards avoiding them.

## **FOLLICULAR-TUBE ABNORMALITIES**

Using slaughterhouse material, it has been demonstrated that the incidence of such abnormalities was very low. However, a 40% incidence has been reported, normally involving adhesions of the infundibulum to other parts of the reproductive tract. Collagenous masses, which may occlude the lumen of the follicular tubule, have been more recently documented. Rarely, an ovarian cyst may be seen to block the entry to the Fallopian tube at the infundibulum. Tumours of the Fallopian tube are extremely rare.

## **UTERINE ABNORMALITIES**

Uterine abnormalities due to congenital defects or infections are relatively well understood in the mare, compared with abnormalities of the remainder of the tract. The development of techniques such as ultrasonic scanning, endoscopy and uterine biopsy has significantly advanced our understanding of uterine physiology and pathology.

**Hypoplasia:** Uterine hypoplasia (underdevelopment) is characterized by an inability to develop adequately in order to maintain a pregnancy. The endometrial glands are those most significantly affected, tending to be very small and so incapable of adaptation to support a pregnancy. As a result, even if fertilization does occur, the EED rate is high. Covering mares too close to puberty is associated with high rates of EED, due to hypoplasia. The actual age at which the uterus is fully mature depends very largely on the individual mare, 18 months to 4 years is considered acceptable. Hypoplasia at 4 years old

or over is indicative of a problem, which is likely to be permanent and may be associated with chromosomal or hormonal abnormalities.

**Hyperplasia:** Uterine hyperplasia (overdevelopment) is characterized by an overdevelopment of the uterus for the reproductive stage of the mare or a failure to recover from a previous event, such as pregnancy. Again, the endometrial glands are most significantly affected. The condition is often a result of delayed involution post-partum. Hyperplasia may also be a result of EED or abortion or a consequence of hormonal imbalance, often resulting from hormone secreting tumours. Hyperplasia is normally a temporary condition, which can be reversed by reproductive rest or hormonal treatment.

**Uterine atrophy:** Mares with uterine atrophy or senility are normally classified as repeat breeders with high rates of EED. Uterine atrophy is caused by a decrease in the number of endometrial glands, due to atrophy or an inability to regenerate. It is often associated with chromosomal intersex conditions, ovarian incompetence or progressive wear and tear in multiparous mares. It is also reported to have a greater occurrence late in the breeding season, presumably due to a decline in oestrous and ovarian activity. This condition is normally irreversible.

**Uterine fibrosis:** Uterine fibrosis is a degenerative uterine change, most commonly found in old multiparous mares; it is characterized by fibrotic changes around the endometrial glands, forming glandular nests.

As a result, the secretions of the endometrial glands decrease and the glands dilate, increasing the incidence of uterine cysts and resulting in increasing EED, due to a disruption of embryo mobility, or abortion in late pregnancy, due to restricted placental size.

**Uterine luminal cysts:** Uterine luminal or endometrial cysts are the most common form of uterine lesion. They are generally thin-walled, greater than 3 cm in diameter and filled with lymph and may occur singly or in multiples. They are particularly evident in mares of 10 years old or over. Their effect on reproductive performance is disputed. If they are present in any number, they are likely to interfere with embryonic mobility, increasing EED, and also to reduce the uterine surface area available for placental attachment, increasing abortion rates. Treatment may be attempted by puncturing the cysts via curettage, endoscopic manipulation or thermocautery, though they may subsequently recur.

**Ventral uterine dilatation:** Ventral uterine dilatation or sacculation is caused by uterine myometrial atrophy, normally in the base of one uterine horn, forming an outfolding or sacculation, which often collects fluid. This is more common in older multiparous mares, due to a weakening of the myometrium. It often occurs at the implantation site, and may be caused by a gradual weakening of the wall in an area of repeated excessive stretching. Treatment is relatively unsuccessful, but some beneficial results have been reported using

oxytocin, or oxytocin in combination with warm saline lavage. The fluid accumulation makes mares susceptible to chronic endometritis and pyometra.

**Uterine adhesions:** Uterine adhesions are present as single or multiple bands or sheets of tissue within or across the lumen of the uterus and are the result of uterine trauma from dystocia, intrauterine infusion or severe endometritis or after treatment with caustic solutions. Their effect upon fertility depends upon their extent, but they may disrupt embryo mobility, restrict placental attachment or even cause post-partum problems, such as placental and fluid retention, leading to endometritis. As such, they may be associated with EED or abortion. Attempts may be made to remove or break adhesions manually via an endoscope and biopsy forceps or electrocautery.

**Uterine neoplasia:** Neoplasia or tumours within the uterus are very rare, but when present can be evident as single or multiple nodules and cause persistent haemorrhage. Treatment may be attempted by surgery or endoscopy with some success.

**Foreign bodies:** Very occasionally, foreign bodies, such as foetal bone, tips of uterine swabs, frozen semen straws, etc., are found within the uterus, resulting in chronic endometritis. Their removal, followed by treatment and recovery time, normally restores reproductive performance.

#### CERVICAL ABNORMALITIES

Cervical abnormalities normally arise from damage at parturition. Lacerations or injuries to the cervix often do not heal properly, thereby causing adhesions, which may block the entrance to the uterus through the cervix or cause cervical incompetence.

This will inhibit sperm deposition and allow infection to enter the uterus. The cervix naturally forms the final seal protecting the upper reproductive tract from infection. Minor adhesions may be treated by physically cutting or electrocauterizing the scar tissue and inserting a plastic tube to prevent reoccurrence, and lacerations can be surgically corrected. The prognosis in most cases, however, is not good. Neoplasms of the cervix are very rare. Inherited cervical incompetence, though, has been reported in pony mares.

#### VAGINAL ABNORMALITIES

Vaginal abnormalities have several causes. Among these is damage at parturition, often a result of foetal malpresentations. Superficial damage will correct and heal naturally, though there is the risk of adhesions. Severe adhesions may cause the mare pain at subsequent coverings.

The prognosis in such cases depends on the length of the opening formed, but can be very poor when substantial rectovaginal fissures occur. Occasionally, a persistent hymen may be evident.

The hymen divides the anterior and posterior vagina and, if not broken prior to the first service, may tear, causing the development of scar tissue. A persistent hymen may also impede natural drainage and lead to pyometra.

Two of the most common vaginal abnormalities are associated with poor perineal conformation, pneumovagina and urinovagina. Pneumovagina (inspiration of air and

bacteria into the vagina) is a common cause of infertility, especially in Thoroughbred mares, and is due to the incompetence of the vestibular and vulval seals and associated poor perineal conformation. This can be alleviated quite successfully by a Caslick's vulvoplasty operation. Urinovagina (urine pooling within the vagina) results from weakness within the vaginal walls and a collection of urine within the resultant sacculation. It is often associated with infection, which can easily spread to the rest of the reproductive tract and hence adversely affect reproductive performance. The condition is normally observed in older multiparous mares, with pendulous reproductive tracts, due to continual stretching and weakening with successive pregnancies. Occasionally, it may also be seen as a temporary phenomenon at foal heat, but in most circumstances it will have rectified itself by the second heat postpartum. If it is evident, it is essential that the mare is not covered, as there is an increased chance of *post coitum* endometritis. Treatment using oxytocin has proved reasonably successful.

#### VULVAL ABNORMALITIES

Vulval abnormalities most commonly involve inappropriate perineal conformation. The resulting vulval-seal incompetence increases the chance of infection entering the reproductive tract. Lacerations of the vulva occurring at parturition or due to accidental injury can again compromise vulval-seal competence, due to incorrect healing and adhesion formation. Failure to cut a Caslick's vulvoplasty prior to covering or parturition will also cause tearing and will predispose to adhesion formation and inappropriate vulval healing. Haemorrhage of the vulval lips may be evident, caused by the bursting of varicose veins. This has a minimal direct effect on reproductive ability but may cause discomfort at breeding. Neoplasms of the vulva are reported, most commonly melanomas, originating in the pigment-producing cells of the skin, especially prevalent in grey mares.

These tumours can spread from the perineal area around the anus and eventually throughout the whole of the body. Squamous-cell carcinoma, normally associated with the penis, may also be seen on the vulval lips. Finally, enlarged clitorises, sometimes in the form of a vestigial penis, may be observed and are associated with chromosomal abnormalities and such animals are sterile.

#### *Infectious infertility*

#### OVARIAN INFECTIONS

The ovary is essentially unaffected and the vast majority of ovarian abnormalities, and hence ovarian infertility, are not the result of pathogenic agents.

#### FOLLICULAR-TUBE INFECTIONS (SALPINGITIS)

Salpingitis, inflammation of the Fallopian tubes or salpinges, is rarely seen, however, it may occur as a consequence of endometritis. Complete blockage of the Fallopian tubes is rare, but inflammation can interrupt the process of fertilization, the passage of ova towards the utero-tubular junction and sperm movement towards the ampulla. Infertility or subfertility may result. Occasionally, infection may cause inflammation of the valve at the utero-tubular junction, affecting the passage of sperm and/or fertilized ova.

## UTERINE INFECTIONS

One of the major causes of infertility in the mare is endometritis. Inflammation of the uterine endometrium, is primarily caused by infection by venereal and opportunistic bacteria. Less commonly, non-infectious degenerative endometritis may occur. The main consequence of endometritis is a uterine environment hostile to embryo survival and implantation, resulting in EED and abortion. Endometritis is evident in four forms: acute endometritis;

chronic endometritis; acute metritis; and pyometra.

There are several factors that predispose the mare's tract to infections, including immunological, physiological or endocrinal deficiencies, which may be inherited, leading to a predisposition to endometritis. Unfortunately, the mare's reproductive tract is not well designed for the easy removal of infective organisms or the resulting exudate. Temporary infertility is nearly always evident with endometritis and, if the infection damage is

great, permanent reduction in reproductive performance will result. Bacterial infection is nearly always introduced at covering or by inadequate hygiene precautions during internal examination or immediately postpartum.

Endometritis is often characterized by excess mucus, which may be seen exuding from the vulva, high leucocyte counts and increased uterine blood flow. Oedema can be identified by scanning and the uterus can be felt, via rectal palpation, to be large and flaccid. The mare may also show shortened oestrous cycles, due to the irritation of the uterine wall resulting in premature CL regression.

### **Potential endometritis-causing bacteria**

There are six major bacteria that cause endometritis, with up to 15 different bacteria identified in some cases. The six major bacteria can be classified as opportunistic or venereal.

#### *Opportunistic bacteria*

They are potential causers of acute endometritis, especially in compromised or susceptible mares. There are three main opportunistic bacteria:

*Streptococcus zooepidemicus* is implicated in 75% of acute endometritis cases, particularly during the initial stages. It has a major role in initiating infection of the mare's cervix and uterus. It may also promote the proliferation of other bacteria within the tract.

*Haemolytic E. coli* is a rod-shaped aerobic bacterium that is found either alone or in short chains. It is the second most common cause of uterine infection. It is naturally found in the intestine and is associated in particular with faecal contamination. It can cause not only acute endometritis but also severe systemic infection, which can prove fatal.

*Staphylococcus aureus* is a less common cause of endometritis. It is a spherical or oval bacterium, normally evident in clusters and found associated with skin and mucous membranes. Under suitable conditions, such as the disruption of the natural microflora, ill health or stress, the bacteria will invade the reproductive tract of the mare.

### *Venereal-disease bacteria*

Venereal-disease bacteria are those that are transferred solely via the venereal route and are capable of producing endometritis in both the normal and the susceptible mare. They may also be present in apparently asymptomatic animals – in particular, the stallion, which rarely shows symptoms. There are three main venereal-disease bacteria:

*Taylorella equigenitalis* is an extremely contagious bacterium and is the causal agent of contagious equine metritis (CEM). It was first isolated in Newmarket, UK, by Crowhurst (1977), where it spread rapidly and widely, due to the reluctance of infected-mare owners not to present their mares for service.

The stallion is seemingly not affected by the bacterium, but is the prime means by which it is spread from mare to mare. In the mare, the typical symptoms of acute endometritis are seen, characterized by uterine, cervical and vaginal inflammation, along with a copious grey discharge within 2–5 days of infection.

In rare instances, the mare may also not show any clinical symptoms but still be a carrier capable of infecting a stallion. At the other extreme, the infection may develop to give chronic endometritis.

*Klebsiella pneumoniae* is an encapsulated rod-shaped bacterium, associated with acute and chronic endometritis. The ones of particular concern are capsular types 1, 2 and 5. The bacteria are endemic and widespread. Unfortunately, the bacteria are relatively insensitive to antibiotics and antiseptic washing agents.

*Pseudomonas aeruginosa*, a slender rod bacterium with rounded ends and flagella, is found widely within the environment. However, some strains of *P. aeruginosa* cause endometritis and may be isolated in stallion's semen or in swabs taken from the urethral fossa, but clinical symptoms are rarely evident. In the mare, *P. aeruginosa* causes a greenish-blue or yellowish-green exudate, which appears to be more prevalent in older mares. It is relatively resistant to antibiotics and antiseptics.

### *Diagnosis*

Due to the highly contagious nature of venereal-disease endometritis, diagnosis and prevention are very important.

Diagnosis of acute endometritis may be obvious due to exudates, or via identification of inflammation via scanning, rectal palpation or endoscopy. Once inflammation has been diagnosed, the causal agent needs to be identified by bacterial culturing. Bacterial culturing may also be carried out as a preventive measure.

Bacterial infections may be identified by swabbing of the reproductive tract. It is normal and recommended practice that swabs are taken from the uterus, cervix, clitoris and urethra opening. Uterine swabbing should be carried out using a guarded swab to prevent contamination *en route* and through an open cervix during oestrus. The other swabs may be taken throughout the mare's oestrous cycle. The resultant swabs are plated out and incubated under varying conditions, normally aerobic and microphilic, to aid bacterial identification. Fungal infections may also be identified in a similar manner. The use of swabbing is a widespread and often compulsory practice. Some breed societies have successfully used it to eradicate specific causes of infection in many areas worldwide.

Uterine aspirations and washings may also be collected, especially if purulent material and fluid are present. Culturing of the washings allows bacteria to be identified.

#### *Acute endometritis*

Acute endometritis is invariably a result of significant bacterial challenge by venereal or opportunistic bacteria. Acute infections develop rapidly, giving immediate symptoms of exudate or pus and irregular oestrous cycles. Internally, it causes deep haemorrhage and degeneration of luminal epithelial cells and, in severe cases, degeneration of the deeper stroma cells, leading to areas of missing endometrium. This may lead to hypertrophy and abscess of uterine glands.

Acute endometritis is a major cause of infertility in the mare, providing a hostile environment for both sperm and embryo survival. Bacteria are introduced into the system at covering or at veterinary inspection. It is now accepted that some degree of acute endometritis is evident after all coverings, regardless of the extent of bacterial invasion.

Normally, post-coital endometritis causes no long-term effect, the mare's system being able to adequately deal with the challenge presented, both via an immunological response and by uterine contractions used to eliminate infections and exudates. In most mares, the inflammatory response subsides within 72 h, and the uterus is able to receive the fertilized ovum.

Treatment for general acute endometritis begins with an attempt to correct any physical abnormalities that may be predisposing the mare to infection, such as a Caslick vulvoplasty, a Pouret operation, removal of adhesions, etc. The existing infection is then treated normally by local antibiotics, systemic antibiotics or uterine lavage.

Post-coital acute endometritis: Post-coital acute endometritis is the specific term given to acute uterine inflammation resulting from covering which is evident, to some extent, in all mares, most especially in susceptible mares.

#### *Chronic endometritis*

It may be divided into chronic infective endometritis and chronic non-infective degenerative endometritis.

Chronic infective endometritis can arise from an untreated or inappropriately treated acute uterine infection or due to a mare's inability to satisfactorily combat the initial infection. The condition is more often found in older multiparous mares. It can be extremely damaging to the uterine tissue, causing degeneration and necrosis and resulting in permanent infertility. Treatment is as indicated for acute endometritis but with particular use of infusion and lavage.

Endometrosis, or chronic non-infective degenerative endometritis is caused by degeneration, rather than by infection of the endometrium, and may be classified as infiltrative or degenerative.

Infiltrative endometrosis may be a result of changes within the uterus due to a busy breeding career, and is associated with a natural increase in leucocyte response to the normal bacterial challenge *post coitum*.

Degenerative endometrosis is a degeneration of the endometrial glands, rendering the uterus incapable of supporting a pregnancy. It is associated with EED and is often the result of repeated gestations, especially in mares with a history of uterine infections. Degeneration of the endometrial glands results in a failure to return to normal post-partum, leaving lymph-filled lesions. The prognosis for such mares is poor.

*Acute metritis:* Acute metritis is potentially the most serious uterine infection. It is associated with a massive contamination of the whole uterus as a result of trauma, often during parturition involving retained placental or foetal tissue, or due to bacterial infection introduced via air inspired post-partum or via hands used to aid parturition. Occasionally, it may be evident *post coitum*. Decomposition of retained tissue encourages rapid bacterial growth, along with toxin production. The inflammation of the entire uterus then favours the passage of toxins into the main circulation, resulting in toxemia and, potentially, death. Treatment must be immediate and normally involves large-volume lavage and possibly oxytocin to encourage uterine contraction and thus the flushing out of the uterine contents. The prognosis is often poor.

*Pyometra:* Pyometra is characterized by fluid accumulation in a large, pendulous uterus. In time, the uterine walls may become leathery, tough and fibrous, due to continual infection. Such mares may appear healthy, but often do not show oestrous cycles, due to the inability of the uterus to produce PGF<sub>2α</sub>. Pyometra may be associated with a blockage of the uterus, fibrosis, adhesions, etc., resulting in a build-up of exudate within the uterus, with no normal drainage.

**Uterine fungal infections:** Mycotic or fungal infections of the endometrium are relatively uncommon in the mare. The most common is *Candida albicans*, a yeast. They disrupt the ability of the uterus to support a developing embryo and, if present later in pregnancy, can cause abortion via placentitis and occasionally foetal infection. The majority of fungal abortions occur around 10 months of pregnancy. Treatment is via infusion of antimycotic agents, such as povidone-iodine or nystatin, but the success rate is low.

**CERVICAL INFECTIONS:** Cervicitis, inflammation of the cervix, is usually associated with and is often the initial cause of endometritis. Such infection causes inflammation and possible pus accumulation.

**VAGINAL INFECTIONS:** Vaginal infections are uncommon, the only one of note being dourine. Dourine is caused by a sexually transmitted protozoan. It causes vaginal and vulval infection and inflammation, along with discharge. If left untreated, it will develop systemically to form raised rings within the mare's coat, along with depigmentation of the genitals, plus fever and death in 50–75% of cases.

**VULVAL INFECTIONS:** Equine coital exanthema, genital horse pox, evident as vesiculation and ulceration of the vulval lips or penis, is caused by EHV3. Infection causes pain at covering and can therefore affect reproductive performance. It is sexually

transmitted and symptomless carriers are reported. Treatment with antibacterial creams or powders prevents secondary infections and helps the natural healing process.

**VIRAL INFECTIONS:** The incidence of viral abortion is 1–5%, mainly occurring in late pregnancy. There are two viruses that have a major effect on reproductive performance in the mare: EVA and EHV. EVA causes abortion in mares. The virus is spread via the respiratory route, via the venereal route and from the placenta of aborting mares. Stallions are the major route of infection. There are four main strains of EHV: 1, 2, 3 and 4. EHV3 is the causal agent for equine coital exanthema, which has been discussed. EHV1 and EHV4 are of concern with regard to reproductive performance, as they can cause abortion. In particular, EHV1 is the causative agent for rhinopneumonitis abortion. Virus transfer is via the respiratory route, from birth fluids, soiled bedding, placental tissue, etc. It may also be found in semen. The virus causes placental separation resulting in foetal suffocation and abortion, with 96% occurring in the last 4 months of pregnancy. It can have a devastating effect, causing abortion storms in mares, plus neonatal losses.

#### *Foetal congenital deformities*

Many foetal developmental deformities have been reported. Many of these are not compatible with foetal life and so cause abortion. Chromosomal defects also occur, leading to EED rather than abortion.

### **EQUINE BREEDING MANAGEMENT**

"Assisted reproduction" or "AR" is the way in which humans intervene in, and hopefully improve, the reproductive processes of many species. These include techniques such as artificial insemination, transported semen, embryo collection and transfer, gamete (sex cell) and embryo preservation, and fertility/infertility management.

AR helps to improve the reproductive efficiency of mares and stallions, allowing them to reproduce more reliably, frequently, safely and over a greater portion of their lifetime.

#### **How can we control a mare's cycle?**

Because the natural onset of cycling in early spring is the result of increased perception of light due to increased day length, we can artificially hasten the onset of cycling by putting a mare "under lights" in early December.

A typical stall lit with a 120-200W bulb at 5:00 am until daylight and from 4:00 pm until 10:00 pm will stimulate early cycling activity. Most mares under this lighting regimen will have regular cycles by early to mid-February.

This allows the mare to be bred for an early January foaling date. Young horses competing in weanling, yearling and two-year-old events are at a distinct advantage if they are born earlier in the year.

### **"Short Cycling"**

This diestrus phase of a mare's cycle can be shortened by approximately one week by administering a luteolytic agent which ablates the corpus luteum, the ovarian gland responsible for progesterone production during diestrus.

This is sometimes done at the mare owner's request in order to shorten a mare's stay at a breeding facility or to synchronize the cycles of several mares so that they may be bred at approximately the same time. Conversely, some mares are given supplemental progesterone during diestrus to prolong diestrus, also to aid in the synchronization of multiple mares or to provide additional hormonal support during early gestation.

### **Induction of Ovulation**

As a dominant ovarian follicle matures during the estrus phase of a mare's cycle, it develops the ability to "see" luteinizing hormone, or LH by developing receptors for this protein hormone. LH is the hormone signal responsible for triggering the final maturational changes which a follicle must undergo prior to ovulation. The egg within the follicle must also undergo changes in order to be capable of normal fertilization and subsequent embryonic development. A mare will naturally produce LH and initiate this process on her own.

Some control can be exerted on this process through the use of human chorionic gonadotropin or "hCG". hCG looks, structurally, like LH and therefore is able to trigger the same physiologic response by binding LH receptors associated with the follicle. A mare will generally ovulate within 36-48 hrs of hCG administration as long as the follicle has developed the receptors to "see" it. This is generally the case after 4 days of estrus and a dominant follicle 35 mm or larger in diameter.

By intervening in the mare's cycle with hCG, we can control when she ovulates to better coincide with a stallion's breeding schedule, a frozen semen breeding protocol and synchronization of several mare's ovulations.

### **Artificial Insemination**

Artificial insemination refers to the process of introducing semen into the uterus manually, without a natural breeding. Semen is collected from a stallion using an artificial vagina (AV) and is transferred into a mare's uterus via a long pipette which is passed through the cervix and into the uterus.

Advantages of an artificial insemination program include:

- 1) decreased risk of injury to mares, stallions and handlers
- 2) ability to breed multiple mares with semen from a single ejaculate
- 3) decreased risk of infection to the mare and stallion
- 4) ability to breed a larger book of mares to a stallion
- 5) semen can be transported to mares off the stallion farm and
- 6) increased pregnancy rates overall

Artificial insemination programs also eliminate the need to breed a mare on an every-other-day basis as is the standard procedure in a "backyard" breeding program. Instead, ultrasound imaging of the mare's reproductive tract is used (ideally, on a daily basis) to follow the progression of follicular development as well as endometrial (uterine lining) changes during estrus. Ultrasound can also be used to detect any uterine or ovarian abnormalities which can then be factored into the management of the cycle. Such information is generally undetectable by rectal palpation. Using this information, possibly in conjunction with the above-mentioned cycle management techniques, ovulation can be predicted and insemination performed shortly before (within 24 hours). Pregnancy rates in a well-managed artificial insemination program typically exceed those obtained in a natural service program due to the higher level of attention paid to the mare's reproductive changes as well as a decreased risk of infection and ability to manage otherwise undetectable pathology. Stallion semen is collected with an artificial vagina (AV) consisting of a rigid cylindrical shell lined with a bladder filled with warm water ending in a sterile collection bottle. Most stallions in an artificial insemination program are trained to mount an artificial mare known as a "phantom" and are collected on an every-other-day basis. A teaser mare is often present to provide additional stimulation for the stallion.

Once collected, the semen should be evaluated for volume, concentration, motility, total spermatozoa count, and overall character. Proper handling of the semen is critical as semen is extremely sensitive to temperature and contamination.

The evaluation procedure allows the breeding manager to determine the necessary volume of semen to be introduced into the mare's uterus. As each collection is different, an evaluation should be performed for every collection. Ideally, a mare should be inseminated with enough semen to provide 500 million progressively motile spermatozoa. Most stallions provide collections yielding 4 to 12 billion spermatozoa, allowing for multiple inseminations.

Before insemination, the semen should be diluted in an "extender" (a nutrient mixture typically formulated from skim milk, glucose and antibiotic) to extend its' longevity and decrease the risk of infection.

### **Transported Semen**

The advent of shipped semen technology has drastically changed the horse breeding business. In years past, a broodmare had to be transported to the stallion in order to be bred. This involved considerable risk, expense and inconvenience to the mare owner. Transported semen, whether cooled or frozen, has allowed the breeder to select from stallions anywhere in the world. As a result, the overall quality of foals produced has improved greatly.

Cooled-shipped semen is collected and evaluated according to the above method and diluted into extenders specially formulated for maintaining the spermatozoa for up to 72 hours. A minimum of 1 billion progressively motile spermatozoa should be provided in

each insemination dose, allowing for some loss during transit. The semen is packaged into a shipping container which has been designed to cool the semen slowly from 37 degrees C to approximately 5 degrees C over a period of 8 hours. The semen is transported to the mare via overnight or same-day delivery and inseminated upon arrival. Insemination should occur within 12-24 hours prior to ovulation. Shipped semen conception rates vary among stallions but are, overall, quite good in a well managed program.

Stallion semen may be frozen and stored at very low temperature for an extended period of time. It is stored submersed in liquid nitrogen at -196 C, a temperature so low that metabolic reactions in the spermatozoa occur at an extremely slow rate. In essence, the spermatozoa are kept in a state of suspended animation. The methods used to freeze equine semen are highly technical, necessitating very specialized equipment and instrumentation. Protocols dictate very precise handling methods, packaging, cooling and thawing rates and temperatures.

Conception rates with frozen semen are generally lower than with fresh or cooled semen. The rigors of the freezing and thawing process invariably result in the loss of a portion of the motile spermatozoa. Post-thaw motility rates in the 40%-50% range are likely, with rates above 60% considered above-average. Also, most frozen semen has a decreased longevity in the mare's reproductive tract after insemination, requiring a more exacting breeding protocol.

Best results are seen when a mare is inseminated within a 4 to 6 hour window around ovulation. hCG is often administered to better control/predict the timing of ovulation. An average of two cycles per pregnancy should be expected, though fertility is variable among stallions.

### **Embryo Transfer**

Embryo transfer (ET) entails the collection of a 7-8 day old embryo from a "donor" mare's uterus and transferring it into the uterus of a reproductively sound "recipient" mare. The cycles of the donor and recipient are synchronized with one another, often through the use of the above-mentioned hormonal modulation techniques. Once in estrus, the donor mare's cycle is not managed any differently than if she was to carry the foal on her own. However, at 7-8 days after ovulation, her uterus is flushed with several liters of an embryo collection medium with the fluid recovered and filtered through a very fine mesh which traps the embryo. At this time point in development, the embryo is an "expanded blastocyst" and is 500-1500 microns (0.5-1.5 mm) in diameter.

The filtered fluid is searched under high magnification for the embryo which is then transferred into a special holding medium, where it will remain until transferred into the recipient. Embryo recovery rates are quite high (approximately 75% per attempt) when a fertile mare is bred to a fertile stallion. Problem mares will yield embryos at a lower rate depending upon their unique reproductive profile.

Once identified, the embryo is transferred, either surgically or non-surgically through the cervix, into the recipient's uterus. Most embryo transfer facilities currently use the non-surgical transfer procedure due to its greater ease and good rate of success (approximately 75-80% per transfer).

Recipient mares are often given supplemental progesterone after the transfer, improving the chances of success.

The recipient mare makes no genetic contribution to the developing foal, though her size can impose limits on the growth of the foal in utero.

Embryo transfer is an excellent means of obtaining multiple foals from a donor mare. "Superovulation" techniques allow breeders to collect and transfer multiple embryos from a mare on a single cycle—2 to 4 on average. ET also allows many mares which are incapable of carrying a pregnancy to term to continue producing. Show mares can produce foals with a minimal layoff from training and showing.

### **Oocyte Transfer**

A variation on the embryo transfer theme is oocyte transfer. Oocyte (egg) collection involves the use of an ultrasound-guided needle to puncture the donor's preovulatory follicle. The contents of the follicle, ideally including the egg, are then aspirated into a collection bottle and searched under high magnification for the oocyte. Equine oocytes are quite small (100 microns or 0.1 mm in diameter) and the search and handling process is a delicate one.

Once recovered, the oocyte is surgically transferred into the oviduct of a synchronized recipient. The recipient has been artificially inseminated with semen from the stallion of choice an hour, or so, before the transfer. The spermatozoa have traveled into the oviduct into which the oocyte is transferred and conception occurs in the recipient. She then carries the developing foal as if it were her own. Again, Superovulation is often employed in order to yield multiple oocytes from the donor.

Equine oocyte transfer is a relatively new offering for the breeder, available commercially since 1995. Pregnancy rates have improved considerably and approach those of embryo transfer. It is a very valuable tool for obtaining foals from mares which are incapable of producing an embryo (due to oviductal blockages) or whose uterine environment is incapable of sustaining an embryo to the 7-8 day time-point when an embryo can be collected.

### **Seasonal breeders**

**Seasonal breeders** are [animal species](#) that successfully mate only during certain times of the year. These times of year allow for the births at a time optimal for the survival of the young in terms of factors such as temperature, food and water. Related [sexual interest and behaviors](#) are expressed and accepted only during this period. Female seasonal breeders will have one or more [estrus cycles](#) only when she is "in season" or fertile and receptive to mating. At other times of the year, they will be anestrus. Similarly, male seasonal breeders may exhibit changes in [testosterone](#) levels, [testes](#) weight and fertility depending on the time of year.

Seasonal breeders are distinct from [opportunistic breeders](#), which mate whenever the conditions of their environment become favorable, and [continuous breeders](#) like humans that mate year-round.

### **Physiology**

The [hypothalamus](#) is considered to be the central control for reproduction. Hence, factors that determine when a seasonal breeder will be ready for mating affect this tissue. This is achieved specifically through changes in the production of the hormone [GnRH](#). GnRH in turn transits to the [pituitary](#) where it promotes the secretion into the bloodstream of the [gonadotropin LH](#), a pituitary [hormone](#) critical for [reproductive function](#) and behavior. Changes in gonadotropin secretion initiate the end of anestrus in females.

### **Factors that determine time of fertility**

#### **Photoperiod**

When a seasonal breeder is ready for mating is strongly regulated by length of day ([photoperiod](#)) and thus season. Photoperiod likely affects the seasonal breeder through changes in [melatonin](#) secretion by the [pineal gland](#) that ultimately alter GnRH release by the hypothalamus.

Hence, seasonal breeders can be divided into groups based on when they are fertile. "Long day" breeders cycle when days get longer (spring) and are anestrus in fall and winter. "Short day" breeders cycle when the length of daylight shortens (fall) and are anestrus in spring and summer. Domestication has allowed cattle and swine to be liberated from breeding seasonality. Day length variations with latitude can also impact breeding. For instance, sheep and goats in tropical climes may breed throughout the year while those in more polar arctic areas may have a shortened season.

Females are generally more sensitive to changes in day length. For instance, unlike mares, stallions remain fertile year-round, suffering only some declines in sexual behavior and spermatogenesis out of season.

#### **Other Factors**

Other factors that affect breeding time include the presence of a ready and available mate. For instance, the presence of a fertile male will induce an estrus cycle in a doe shortly after introduction.

Further environmental factors can include nutrition, chemosensory and hormonal cues. Weight and age are other factors.

### **Partial list of seasonal breeders**

Many non-mammals are seasonal breeders, such as many [birds](#) and [fish](#). Here is partially listed those that are mammals.

#### **Long day breeders**

[Ring-tailed lemur](#)

[Horse](#)

[Hamster](#)

[Groundhog](#)

[Mink](#)

#### **Short day breeders**

[Sheep](#)

[Goat](#)

[Fox](#)

[Deer](#), [Red Deer](#)

[Elk](#)

[Moose](#)

#### **Summer breeders**

[Ruffed lemur](#) (May - July)

Select [species](#) of [hamster](#), [vole](#) and [mouse](#)

### **Melatonin and Seasonal breeders**

Melatonin, an indolamine, is produced by the pineal gland. Its level of secretion is influenced by the photoperiod, with reducing day length stimulating and increasing day length inhibiting its secretion. Melatonin modulates either directly or indirectly, the frequency of GnRH secretion from the hypothalamus, thus influencing the secretion of gonadotrophins and cyclical ovarian activity.

#### **Indications**

Advancing the onset of normal cyclical ovarian activity in pure and cross bred lowland breeds of sheep so that early lambing occurs.

In horses Melatonin has been used to **simulate short day cycles**

Melatonin, if given in mid summer, shortens estrous and hastens winter anestrus. Once properly exposed to short days, the mare can then respond to lights or the stimulus of long days. This therapy is considered if the mare does not get pregnant in the summer. If such a mare was given Melatonin at that time, followed by lights next November, regular cyclic activity could be achieved early next season.

"Melatonin is the primary endocrine signal in the horse that produces the effects of season on other parts of the body e.g. reproductive cycle. The circulating melatonin level

is highly dependent on light, it increases in concentration during periods of darkness (night). Seasonal head shakers (spring onset) can be treated with melatonin. Daily administration of melatonin mimics the winter conditions of melatonin kinetics and thereby may decrease the seasonal body changes which lead to the development of **headshaking**."

#### **Dose rate and treatment regimen**

One implant (18mg of melatonin) per ewe inserted subcutaneously on the Outer aspect of the base of the ear. The earliest time of use of implants is determined by decreasing by the breed of ewe. It can also be used in goats. It is critical to ensure that ewes (does) are out of sight, sound and smell of rams (bucks) for at least 7 days before and at least 30 days after the implant is inserted.

#### **Equine cycle seasonality and aberrations**

It has long been recognized that reproductive function of the mare, specifically normal cyclic activity is governed predominantly by photoperiod or in other words day length. To varying degrees, mares naturally respond to changes in day length and generally experience:

- 1) Autumnal transition
- 2) Winter anoestrus
- 3) Vernal transition (these are collectively termed the Anovulatory season)
- 4) Seasonal polyestrous cyclicity (breeding or ovulatory season) during late spring and summer.

Photoperiod influences ovarian at least in part through the actions of melatonin. Melatonin is synthesized and secreted in the pineal gland mostly at night and is thought to act by decreasing the synthesis of GnRH in the hypothalamus. Decreasing day length in the fall results in lower hypothalamic GnRH content and reduced secretion of LH and FSH by the adenohypophysis. Clinically, mares may have reduced fertility and persistent inoculator follicles and possibly prolonged periods of estrus during the autumnal transition (receding phase). During the winter, hypothalamic GnRH is at its lowest concentrations consistent with short photoperiods and a high level of pinealocyte melatonin synthesis. Owing to the absence of GnRH activation of the promoter for the LH  $\beta$  subunit, the adenohypophysial content of LH is minimal during anoestrus. The concurrent decreases in ovarian activity and size are accompanied by passive sexual behaviour in anoestrous mares. Increasing photoperiod after the winter solstice results in a gradual decrease in melatonin synthesis and an increase in pulsatile secretion of GnRH. FSH secretion is subsequently stimulated and folliculogenesis is initiated. Tentative lack of LH secretion is evidenced by the development of multiple transitional, inoculators follicles. The ovaries of mares in the vernal transition (resurging phase) are frequently described as resembling 'cluster grapes' by transrectal palpation. Sufficient GnRH stimulation induces LH synthesis and secretion and ovulation. Subsequent to this initial ovulation in a previously anovulatory mare, the sequential and cyclic endocrine and functional reproductive events which characterise the equine breeding season take place. Several photoperiod manipulation techniques have been employed to hasten the onset of ovulatory estrous cycles in mares. Exposing mares to 16 hours of artificial light (10-12

foot-candle of light) or increasing day length after sunset for 2 to 3 hours for a total photoperiod of 16 hours have been used successfully to manipulate photoperiod. The introduction of an hour of light 9.5 hours after the onset of darkness has been suggested as another alternative to stimulate mares to cycle earlier in the year. Even with artificial photo stimulation, mares will experience a “transitional” phase. Photoperiod manipulation schemes should commence at least 60 days before the desired time of breeding.

### **Mare's estrous cyclic peculiarity**

The normal estrous cycle of the mare is approximately 21 days and normal length of estrus and the interjectors interval range from 5-7 days and from 14-16 days respectively. The estrous cycle of non pregnant mares is characterized by a typical pattern of hormone concentration in the peripheral blood. Individual variations in the estrous cycles exist and dependent on:

- 1) Seasonal influence
- 2) Body condition
- 3) Exposure to ergot alkaloids in hay
- 4) Number of follicular waves
- 5) Possibility of multiple or diestrous ovulation

The normal estradiol of follicular origin is the predominant reproductive steroid in the blood at the beginning of estrus. Progesterone (P4) is low at this time and mare shows behavioral signs of estrus when teased (eversion of clitoris, frequent urination, standing for stallion). Dominant follicle grows in size and produce more estradiol. Subordinate follicles become atretic owing to production of inhibin (induced by oestradiol) by granulosa cells of the dominant follicle. Under the influence of GnRH, pulse frequency favours secretion of LH relative to FSH. LH levels increases and FSH reaching minimum levels. The revelatory Lh surge is more gradual in the horse as compared to other species and actually reaches maximum peripheral blood concentration 1-2 days following ovulation. As LH levels increases and the follicle approaches ovulation, theca cells begin to degenerate and oestradiol production actually begins to decrease prior to ovulation. Blood levels of follicular oestradiol and inhibin return to their basal levels over a 1-2 days period following ovulation and this decline in oestradiol coincides with the end of behavioral estrus in the mare. Abnormal prolongation or increases frequency of oestus is caused by:

- 1) Short interjectors interval leading to early return to estrus
- 2) Large quantities of oestrogen synthesis during pregnancy leading to exhibition of prolonged estrus.
- 3) Granulation cell tumours
- 4) Failure of older mares or mares during vernal transition to ovulate.

Dioestrus begin following ovulation with cessation of estrous behaviour. Predominant hormone during this period is progesterone (P4). Under the influence of P4, GnRh pulses are infrequent and FSH pulse amplitude increases relative to LH. Peripheral blood levels of FSH reach a maximum during diestrus allowing for follicular development.

## **Pecularity of Swine Breeding and Reproductive Performance**

Puberty in gilts is reached at about 7 months, but diet, breed (including degree of inbreeding) and season of birth influence its onset. At the first estrus, the number of ovulations is low but it increases thereafter so that if mating is delayed until the 3<sup>rd</sup> heat a large litter will result. The cross breeding of inbred lines increases the ovulation rate, as does the provision of a high energy diet for 11-14 days before the expected estrus. Fecundity is best from 4<sup>th</sup> to 7<sup>th</sup> gestation. Domestic sow is generally polyestrous but wild pig is seasonal breeder. Inter-estrous interval is 21 days interrupted by

1) Pregnancy

2) Lactation

Average length of estrus is 53 hrs (48-72 hrs) with spontaneous ovulation occurring between 38 and 42 hrs from the beginning of estrus. Best breeding time is therefore approximately 24hrs after onset of estrus. During lactation, the physical stimuli of suckling suppresses cyclical activity but many sows show an oestrous estrus 2 days after farrowing. When weaning occurs at 5-6 weeks, estrus can be expected in 4-6 days. Earlier weaning results in a slightly longer time interval.

### **Signs of Oestrus**

1) Progressive swollen and congested vulva

2) Restlessness

3) Repetitive grunt emission

4) Assumes a stationary rigid attitude with cocked ears

5) Remains still during coitus

### **Methods of Heat Detection**

#### **Cattle**

1) Use of Heat mount detectors

2) Use of closed circuit television

3) Use of progesterone assay

4) Use of teaser bull

5) Use of androgenised cows

6) Use of Oestrus synchronisation and induction programmes

#### **Sheep**

1) Use of vasectomised ram

2) Use of androgenised female

3) Estrous behaviour

#### **Goat**

1) Use of 'buck rag'

2) Use of vasectomised bucks

3) Use of intersex or hermaphrodite

4) Use of androgenised females

5) Estrous behaviour

## **Artificial Insemination time**

### **Cow**

Ovulate at about 10-12 hours after the end of the estrus period.

Estrus Duration - 18hrs

Ideal Insemination time is about 6 - 24 hrs prior to ovulation. Some prefers - from midestrus to 6hrs after end of estrus. In practice, optimum insemination times achieved are on the morning of the next day when estrus is first observed in the afternoon.

### **Ewe**

Ovulate at about the end of estrus

Estrus Duration - 24-48hrs

Ideal insemination time- Before ovulation. 18-20 hrs after onset of estrus. Highest conception rates are therefore achieved when the timing of insemination is optimized by drafting ewes for insemination twice per day.

### **Goat**

Ovulate (spontaneously) at about 12-36 hours after onset of standing heat could be hastened by presence of male.

Estrus duration - 12-24 hrs

Ideal insemination time -Preference is for 12 hrs after first observation of heat

### **Sow**

Ovulate at about 16-18 hrs after onset of heat for estrus duration of 24hrs but 48-54 hrs after onset of estrus for estrus duration of 72hrs.

Estrus duration - 24 - 72 hrs

Ideal Insemination time - Approximately 24 hrs after onset of estrus.

## **Improvement on GnRH- PGF<sub>2</sub> $\alpha$ protocol – MELENGESTROL ACETATE PROGRAM**

Use of MGA to control estrous cycles of cows or heifers in breeding programs involving natural service or artificial insemination has evolved with time. Four methods for using MGA are available to facilitate estrus synchronisation in heifers or cows. MGA may be fed as premix with a grain or protein carrier and either top-dressed onto other feed or atch- mixed with larger quantities of feed. MGA is fed at a rate of 0.5mg/animal/day in one feeding. Cows and heifers will exhibit estrus beginning 48 hours after MGA withdrawal, and this behaviour will continue for 6 to 7 days. It generally is recommended that females exhibiting estrus during this period not be inseminated or exposed to natural mating because of the reduced fertility among such animals at first heat after MGA withdrawal

## Methods

MGA with Natural mating - the simplest MGA-based method of estrus synch involves using bulls to breed synchronized groups of females. Cows and heifers receive the normal 14 day feeding period of MGA and are then exposed to fertile bulls about 10 days after MGA withdrawal. A ratio of 15 -20 synchronised females per bull is recommended  
MGAplus Prostaglandin: here  $\text{PGF}_2\alpha$  is administered 19 days after the last day of MGA feeding. This treatment places all the animals in the late luteal stage of the oestrous cycle at the time of injection, which shortens the synchronised period and maximizes conception rate.

MGA Select: this involves feeding MGA for 14 days, followed by an injection of GnRH on day 26 and an injection of  $\text{PGF}_2\alpha$  on day 33. The addition of GnRH to the 14 to 19-day MGA-  $\text{PGF}_2\alpha$  protocol improves synchrony of estrus while maintaining high fertility in postpartum beef cows

7-11 synch: this is aimed at 1) shortening the feeding period of MGA without compromising fertility and 2) improving synchrony of estrus by synchronizing development and ovulation of follicles from the first wave of development. here, MGA is fed for 7 days then animals treated with  $\text{PGF}_2\alpha$ .  $\text{PGF}_2\alpha$  4 days after they are treated with GnRH and 7 days after with  $\text{PGF}_2\alpha$

An additional consideration pertains to cows or heifers that fail to exhibit estrus after the last  $\text{PGF}_2\alpha$  injection. Such cows or heifers can be reinjected with  $\text{PGF}_2\alpha$  11 to 14 days after the last injection of  $\text{PGF}_2\alpha$  was administered. These females are then observed for signs of behavioural estrus for an additional 6 to 7 days. This procedure maximizes the number of females inseminated during the first 2 weeks of the breeding period.

### Intravaginal progesterone releasing insert

The presence of anestrus beef heifers and cows at the start of the breeding season is a major limitation to the success of a  $\text{PGF}_2\alpha$  -based estrus synchronization program. Administration of a progestogen improves estrus synchronisation results through the induction of estrus and ovulation, as well as by providing time for corpora lutea to become responsive to the luteolytic action of  $\text{PGF}_2\alpha$  (> 5 days after estrus; day 0 = estrus). Some of these are applied with a PRID or CIDR, for 8 -10 days followed by the administration of  $\text{PGF}_2\alpha$  or analogue on the day before withdrawal. Such regimens will result in 95% of cows being in oestrus within 5 days

## Disturbances of Bovine oestrus cycle

### Congenital

#### Freemartinism –

The freemartin is the female member of the bovine mixed- sex twins in which interplacental blood vessel anastomoses causes chromosomal chimera and prevent the normal development of genital organ. The interchorial anastomosis develop between day 30 and 40 of pregnancy in the cow when the gonadal ridge in the female has not yet

differentiated into ovary. From the testes of the male twin, 2 different factors are transfused to the female foetus, a steroidal factor stimulating the growth of the wolffian duct (WSF) and a non androgenic polypeptide factor inhibiting the mullerian duct (MIF). The suppression and masculinisation of the ovary and the whole genital tract requires anastomosis at an early stage of development and no neutralizing enzymes in the placenta. The free martin is completely sterile, while the chimeric male twin is normally fertile. Intersexes can also occur as a result of freemartinism in the goat where placenta fusion occurs in twins of dissimilar sex.

### Symptoms and diagnosis

The suppression of mullerian duct development may vary widely in degree. The abnormally small narrow vulva is readily noticed and the absence of normal vagina and cervix detected by examination with a speculum. The enlargement of the clitoris may be found. Rectal exam reveals the absence of a normal uterus. In the majority of cases only the small firm rudimentary gonads can be palpated at approximately the same size as the ovaries of the normal heifer and connected by a strand of tissue running in an arc across the pelvis. In newborn heterosexual twin calves and also at a more advanced stage, the diagnosis of freemartinism can be confirmed by the recognition of erythrocyte chimerism i.e. identical blood groups, partial haemolysis and agglutination. It should be noted that apparently, single freemartin may occur if the male twin dies in utero. Some sporadic cases of abnormal genital structures in heifer are the results of this

Question: why do we not have freemartinism in other species of animal?

ANSWER: it occurs also in sheep (about 0.8%) and goat (very rarely). In the carnivores it is absent. The reason for this is because the relationship between the fetal membranes of contiguous fetuses is simplest in the carnivore, in which, although the extremities of the allantochorionic sacs impinge on each other, they remain separate. The next gradation is in the mare pregnant with twins; here apparently owing to the lack of uterine length, the distal pole of one allantochorion invaginates the proximal extremity of the other. This results in an unequal sharing of the uterine space and is the reason for the commonly observed disparity of twin foals. In the majority of the twin and triplet pregnancies of sheep and cattle, contiguous chorionic sacs coalesce and in many cases the allantoic vascular intercommunication is the rule. Such anastomosis is present between bovine fetuses from the 40<sup>th</sup> day and forms the main premise of the theory of origin of the bovine freemartinism.

### **Segmented aplasia of the mullerian ducts**

This occurs in the cattle and pigs. The condition is typified early by the arrest of embryonic development of the anterior vagina, cervix and uterus. In some cases the vaginal canal may be obstructed by an abnormally developed hymen. In cattle the condition is common in the white short horns heifers and therefore came to be known as

white heifer disease. It also occurs in roaned and occasionally in red and white short horns and in other breeds and crosses.

It has been suggested that approximately, 10% of white short horns are affected. The mullerian ducts do not develop normally. The degree of hypoplasia differs giving rise to a variety of anomalies of the fallopian tubes, uterus, cervix and vagina. This anomalous development is associated with the recessive gene for white coat colour in females. Where portions of the tubular genitalia are absent, the other portions are connected together by fibro muscular strands. Various portions of the uterus and vagina thus do not communicate with one another or the exterior. As a result the occluded portions become distended by retained secretion. The ovaries and vulva are normal, and normal cyclical changes take place in the ovaries of mature adults.

Symptoms and diagnosis usually apparent is general systemic disturbance sometimes following service and associated with vaginal bleeding very often, the condition is only detected when repeated sterile services are investigated. Rectal exam reveals absence of various portions of the uterus with enlargement of the occluded parts to a varied degree where the hymen is completely imperforate and the anterior vagina patent there is usually massive swelling of the vagina between the cervix and hymen and somewhat lesser uterine distention from the accumulation of fluid. Vaginal exam will show the hymen just beyond the external urinary meatus with some bruising and laceration if service has taken place. The condition simulates pregnancy which must be carefully differentiated if necessary by repeated exam. The condition is differentiated from freemartin by the presence of normal ovaries and the normal anatomy of the vulva and clitoris.

### **Treatment and control**

The likelihood of normal pregnancy is always poor. Where the apparent lesion is the absence of one uterine horn, fertility should theoretically be reduced by 50%. But in many cases, other concurrent non-detectable sterilizing anomalies probably occur. Where there is violent straining as a result of the accumulation of fluid anterior to the hymen, immediate alleviative measures are necessary but the center of the hymen should be located and perforated with a large trochar and cannula. On rare occasions the aperture so made will remain patent and normal fertility follow provided that the remainder of the genitalia is normal. Treatment of other forms of the disease is impossible. Control is difficult except by the restriction of mating of males known to carry the defect. It is a wise precaution to examine all white short horn heifers at breeding age to see whether their genital organs are normal or not.

### **Ovarian hypoplasia –**

One or both ovaries are found missing. Single Recessive autosomal gene is found to be the cause, the expression of which depends on the rest of the genetic background. There is also an association with the degree of white coat colour which could imply that different coat colour genes are in some way involved perhaps having a pleiotropic effect on the developing ovary. It is a common congenital defect. Diagnosis is achieved through breeding records of infertility and rectal examination. It is usually not associated with

other defects but at times with freemartinism. In about 85% of cases hypoplasia only affects the left ovary.

Bilateral hypoplasia results in permanent anoestrus, hypoplasia of the genital tract and an asexual phenotype. Females suffering from unilateral gonadal hypoplasia can be fertile and therefore perpetuate the genetic liability.

#### Ovarian aplasia

One or both ovaries are found missing. Etiology is unknown and frequency is low. It rarely occurs. Diagnosis is by rectal examination and is usually associated with other extensive defects.

#### Nutritional cause

The interrelationships between nutrition and reproduction are among the most important, and probably the least understood of the factors that affect reproduction. Anovulatory anoestrus is a well recognized consequence of deficiencies of both macro- and micronutrients.

**Energy:** the most severe effect of inadequate nutrition is the cessation of cyclical activity although less severe manifestations are silent oestrus, ovulatory defects, fertilization failure and embryonic death. Calving to first service interval is prolonged in cows with fatty liver (mostly seen in highly yielding dairy which are overweight at calving, have poor appetite and consequentially mobilize body fat reserves to meet their energy deficit for lactation). This is mainly due to a delay in the time to first postpartum ovulations which in cows with moderate and severe fatty liver, has been shown to be delayed. Other evidence of impaired reproductive function in cows with mild and moderate fatty liver is a shorter interval between the first and second ovulations. For the cows with fatty liver, the average interval was 16 days, compared with 21 days in the other cows.

**Cobalt** - deficiency usually causes anaemia, inappetance, poor bodily condition, ill thrift and loss of condition. Poor fertility may be present at the time as these obvious signs of deficiency. As with many supposed traced element deficiencies, it exerts its effect upon fertility in a number of silent oestruses, poor pregnancy rates and irregular interoestrus intervals. Sometimes poor fertility in apparently normal cows can be corrected following cobalt supplementation. Deficiency occurs when diets contain 0.07mg/kg D>M> cobalt and is due to failure of vitamin B12 synthesis, which is an essential cofactor for carbohydrate metabolism. The only accurate diagnostic procedure is the estimation of liver vitamin B12.

**Copper** – Copper deficiency has been shown to cause delayed puberty, anoestrus, suboestrus or poor pregnancy rates. When this occurs in association with other signs of hypocuprosis, such as anaemia, poor growth, bleached coat colour and diarrhea a diagnosis is likely.

Manganese – manganese has a ubiquitous role in reproductive function, being involved in steroid synthesis. Both the pituitary gland and ovaries are relatively rich in this trace element. A variety of reproductive disorders which depress fertility in cows have been blamed on manganese deficiency; these include anoestrus, poor follicular development, delayed ovulation, silent oestrus and reduced conception rates. It also causes joint deformity in calves. Under normal circumstances it is likely that normal pasture will provide the necessary requirement of 80mg/kg D.M. in the food although some foods are low in manganese. Manganese is a cofactor in a number of enzymes that are responsible for gluconeogenesis and has a significant role as antioxidant. Manganese is also involved in cholesterol synthesis and, hence affects steroidogenesis.

Others are Vitamin A and  $\beta$  – carotene,

Seasonal – the cow is non seasonal polycyclic animal. The recurring cyclic activity is interrupted by pregnancy, lactation and pathological conditions. In those species which are seasonally polycyclic, the mare, ewe, doe (or nanny goat) and cat, or monocyclic like the bitch, there are periods of sexual quiescence or anoestrus.

Anoestrus - this is an apparent period of sexual quiescence in which there is complete absence of sexual activity and no manifestation of heat. It is the most common single cause of infertility in cattle. Cattle are normally anoestrus before puberty, during pregnancy and for a short period after parturition.

Cows and heifers that are anoestrus can be divided into 2 distinct classes:

Class I: Anoestrus cows where you have functional Corpus luteum (CL)

Class II: Anoestrus cows where you have non functional CL

Prolonged interoestrus interval

Prolonged interoestrus intervals i.e > 24days, are due to the following:

failure to detect oestrus. In these cases the interval will be a multiple of 18 – 24 days, viz. 36 -48 days where one oestrus is missed or perhaps 54 -72 days if two are missed. The genital tract and ovaries will be normal on clinical examination. If only a small number of animals are involved then oestrus can be induced with  $\text{PGF}_2\alpha$  or with Progesterone-releasing intravaginal device (PRID). If large numbers are involved then attention must be paid to improving oestrus-detection

incorrect identification of oestrus. The cow has been in oestrus but not observed, and then has been incorrectly identified at some stage in the subsequent dioestrus.

Intervals will vary between 25 and 35 days or 49 and 532 days. individual cows

can be treated with  $\text{PGF}_2\alpha$  or analogues or with PRIDs. If large numbers are involved oestrus- detection must be improved.

Late embryonic or early foetal death: About 10% of late embryos die between 14 and 42 days; a smaller percentage of early fetal death occurs after this stage. In all cases the life span of corpus luteum is extended, hence the prolonged interval between successive heats. The causes are the same as those described as being responsible for early embryonic death and in most cases no specific cause can be found. If large numbers of animals in a herd are involved and if natural service is used and cows have a history of a mucopurulent vulval discharge or abortions, then *Campylobacter fetus* infection should be suspected and further investigations performed to confirm its absence or presence.

Breeding soundness of a cow

The following are considered in determining the breeding soundness of a cow.

Identity, Identification of species, Identification of breed, Identification of Animal,  
Name of owner

Breeding Record (History)-

Source of Animal

Prevalent disease/ condition at source

Age

Weight

Number in group

Number with health problem

History of the progeny

Physical Examination

Examination from a distance

Examination of the locomotory system

Pulse rate

Heart rate

Respiratory rate

Examination of mucous membrane, examination for wounds, and ectoparasites

Examination of the pelvic region (Ligaments)

Examination of the Genitalia

vulva

vagina

cervix-external os

length

breadth

uterus – tonicity

abnormalities

right and left ovaries – length

breadth

Thickness

Cysts and types

6) ovarian location and structures

7) oviducts

Examination of the mammary gland

Udder - Length

Size

Consistency

Milk examination

Teat - Length

Breadth abnormality