What is a hormone?

A hormone is a chemical messenger that coordinates the activities of different cells in a multicellular organism. The classical definition of a hormone is a chemical substance that is synthesized by particular endocrine glands and then enters the bloodstream to be carried to a target tissue, which has specific receptors that bind it. Neuroendocrine hormones are synthesized by nervous tissue and carried in the blood to the target tissue; for example, the various releasing factors that are produced in the hypothalamus, which travel to the anterior pituitary via the hypothalamus–pituitary blood portal system. Neurocrine hormones are released into the synaptic cleft by neurones that are in contact with the target cells. Paracrine hormones diffuse to neighbouring cells, while autocrine hormones feed back on the cell of origin in a form of self-regulation. At the other extreme, pheromones are produced by one animal and released into the environment to be received by other animals.

Classical Definition of a Hormone: Physiological organic substance produced by certain specialized cells and released into circulating blood or lymph for transport to target tissues where it either stimulates or inhibits function.

Hormones are signal molecules that:
- Are synthesized by endocrine cells
- Are secreted into the circulation
- Interact with proteins called receptors
- Have specific effects on target cells

Modern Definition of Hormone:
Substance released by one cell to regulate another cell. Synonymous with chemical messenger. Delivered through endocrine, neuroendocrine, neurocrine, paracrine, autocrine or pheromonal systems

Why are hormones necessary?

Hormones are involved in maintaining homeostasis – consistency of the internal environment that is maintained for the benefit of the whole organism. Claude Bernard first recognized homeostasis in the 19th century, who noted that the internal environment (i.e. the fluid bathing cells) had to be regulated independently of external environment. Being able to regulate and maintain its internal environment gives the
animal freedom from changes in the external particular level. The set point of the system can be altered to affect the levels of the metabolite by altering the sensitivity of the target tissue to the hormone or the sensitivity of the endocrine gland to negative feedback from the metabolite. In addition to maintaining homeostasis, hormones can also be used to drive change in an organism. In this case, levels of hormone increase to some peak, and this occurs by positive feedback. Positive feedback amplifies the response, so the tissue must be desensitized or turned over to stop the response. An example of this response is the surge of luteinizing hormone (LH) that leads to ovulation.

**How do hormones function?**
Hormones cause a trigger effect to modulate the activity of the target tissue. The effects of hormones are seen long after levels of the hormone return to basal values. In contrast, nervous signals are short lasting and more immediate. However, nervous signals can regulate hormone production as well. Hormones are present in trace amounts in plasma, usually ranging from $10^{-9}$ to $10^{-6}$ g ml$^{-1}$. They are present at all times in order to maintain receptors in the target tissue and keep the tissue primed for a response. Hormones are secreted in variable amounts according to need, and there is a constant turnover by inactivation and excretion of the hormone.

**What effects are due to hormones?**
Hormones cause changes in cellular metabolism, but they do not make a cell do something it was not previously capable to do. Hormones do not directly cause changes in gene structure but can activate genes to influence gene expression and ultimately protein synthesis. Hormones can alter catalytic rates of enzymes, by mechanisms such as the phosphorylation and dephosphorylation of proteins. Hormones can also alter membrane permeability to affect transport processes and ion movements, alter muscle contraction, exocrine secretion and water permeability.

Hormones can:
- cause morphological changes, such as the differences in body shape between males and females;
- act as nitrogen to accelerate cell division or alter gene expression to trigger differentiation of cells;
- stimulate the overall rate of protein synthesis or the synthesis of specific proteins;
- be involved in stimulating smooth muscle contraction; for example, oxytocin stimulates contraction of the myoepithelium in the mammary gland for milk ejection;
- affect exocrine secretions; for example, secretin, a peptide hormone from intestinal mucosa stimulates pancreatic secretions;

![Anatomical Relationship of Pituitary to Hypothalamus](image-url)
control endocrine secretions, and a number of trophic hormones from the anterior pituitary can stimulate or inhibit hormone secretion from target organs;

regulate ion movements across membranes and control permeability to water; for example, antidiuretic hormone (ADH, vasopressin) increases water reabsorption by the kidney; and

have a dramatic effect on behaviour, such as sex-related behavioral characteristics, maternal behavior, nesting activity and broodiness.

**How is hormone action selective?**
The method of hormone delivery to the target cells and the presence of specific receptors in the target cells determine the selectivity of hormone action. For example, the hypothalamic–portal system linking the hypothalamus to the pituitary delivers releasing hormones from the hypothalamus directly to the target cells in the anterior pituitary. Smaller quantities of hormones are needed since there is less dilution of the hormone in selective delivery systems compared to hormones that reach their target via the peripheral circulation. Many hormones are linked to carrier proteins in the blood, which stabilize the hormone and increase its half-life in the circulation. For example, sex hormone binding globulin is synthesized in the liver and binds testosterone and estradiol in the circulation with a high affinity.

However, the main factor that determines the sensitivity of a particular tissue to a hormone is whether the tissue contains the specific receptor for the hormone – the tissue will not respond to the hormone unless it has enough of the specific receptor. Receptors are specific proteins present in target cells that bind a particular hormone and initiate a response. Receptors are normally present in small numbers (10,000 molecules per cell). There are two general types, cell-surface receptors and intracellular receptors. Peptide and protein hormones generally do not enter the cell, but interact with cell-surface receptors. For some cell-surface receptors, a second messenger system is needed to transmit the hormone response signal from the outside to the inside of the cell. This involves the activation of a protein kinase, which phosphorylates specific proteins within the cell to alter their function. Steroid hormones and thyroid hormones enter the cell to interact with intracellular receptors and regulate gene expression.
Types of hormones:
The major structural groups of hormones are
• Steroids;
• Proteins, polypeptides and glycoproteins;
• Amino-acid derivatives (especially derivatives of tyrosine);
• Fatty acids and derivatives, such as prostaglandins.

Synthesis of protein hormones:
Peptide and protein hormones consist of a linear chain of amino acids. As with any protein, the specific sequence of the different amino acids determines the primary structure and nature of the protein. The amino acid sequence information for a protein is contained in the sequence of bases (A,C,G,T) in the coding region of the gene that codes for the protein. A three-base sequence codes for one amino acid; this is known as the genetic code. This code is copied from DNA into mRNA by transcription and the mRNA is used to direct protein synthesis by the process of translation. Signal peptides are short sequences of 15–30 hydrophobic amino acids located at the amino-terminal (beginning) of proteins. The presence of the signal sequence (S) directs the newly synthesized protein into the endoplasmic reticulum and then to export from the cell. Other proteins enter the cytosol and from there are directed to the mitochondria (M) or nucleus (N), or to other sites within the cell. Proteins move between the various compartments by vesicular transport. The uptake of proteins by particular vesicles is controlled by the sorting signal sequences in the proteins. Newly synthesized protein hormones containing signal sequences are known as prehormones. Some peptide hormones are synthesized as part of a larger precursor, called a prohormone. Examples of prohormones include proparathyroid hormone, the precursor of parathyroid hormone and proinsulin, which is the precursor of insulin. Proopiocortin is the precursor of several trophic peptide hormones produced in the anterior pituitary. The newly synthesized prohormone with a signal peptide is known as a preprohormone. A number of hormones, including thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH) and LH, have sugar units attached to the amino-acid side chains, and are known as glycoproteins. After protein synthesis, the preprohormone moves from the endoplasmic reticulum to the Golgi apparatus, where sugar residues are attached to asparagine, serine and other amino-acid side-chains in a process called glycosylation. These sugar units can form complex branched chains. From the Golgi apparatus, the proteins are packaged into secretory vesicles and the active hormone is generated by cleavage of the prohormone.
sequences. The secretory granules fuse with the plasma membrane to release their contents by exocytosis when the cell is stimulated.

**Synthesis of steroid hormones:**

**Steroid hormones** are produced in the gonads and the adrenal gland. The gonadal steroids include the *progestins*, *oestrogens* and *androgens*. The adrenal cortex produces *glucocorticoids* and *mineralocorticoids*. Cortisol, a major glucocorticoid, promotes gluconeogenesis and fat and protein degradation. Aldosterone, a major mineralocorticoid, increases absorption of sodium, chloride and bicarbonate by the kidney to increase blood volume and blood pressure. The synthesis of steroid hormones occurs on the smooth endoplasmic reticulum and in the adrenal mitochondria. Cholesterol is the precursor of all steroid hormones and is present as low-density lipoprotein (LDL) in plasma. Many of the steps in the biosynthesis of steroids involve an electron transport chain in which cytochrome P450 is the terminal electron acceptor and carries out hydroxylation reactions. The conversion of cholesterol to pregnenolone involves removal of the C6 side chain from cholesterol by hydroxylation at C20 and C22 and cleavage of this bond by *desmolase* (P450 side-chain cleavage). This step occurs in adrenal mitochondria and is stimulated by adrenocorticotropic hormone (ACTH). Pregnenolone is then converted to progesterone by oxidation of the 3-hydroxy to a 3-keto group and isomerization of the Δ5 double bond to a Δ4 double bond. Progesterone is converted to cortisol by hydroxylation at C17, C21 and C11. Progesterone is converted to aldosterone by hydroxylation at C21 and C11, and oxidation of the C18 methyl to an aldehyde. Progesterone is converted into androgens by hydroxylation at C17 and cleavage of the side-chain to form androstenedione (or androgen). The 17-keto group is reduced to a hydroxyl to form testosterone. Androgens are converted into oestrogens by loss of the C19 methyl group and aromatization of the A ring. The formation of oestrogens from androgens is catalysed by the aromatase enzyme CYP19.

**Synthesis of eicosanoids**

The *eicosanoid hormones* include *prostaglandins*, *prostacyclins*, *thromboxanes* and *leukotrienes*. They are locally produced within cell membranes and have autocrine and paracrine effects. They stimulate inflammation, regulate blood flow and blood pressure, affect ion transport and modulate synaptic transmission. They are synthesized from 20 carbon fatty acids, such as arachidonic acid (20:4) derived from membrane lipids. The enzyme *cyclooxygenase* (COX) catalyses the first step in the conversion of arachidonate to prostaglandins and thromboxanes. Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen and acetylsalicylic acid, inhibit COX and reduce the production of prostaglandins and thromboxanes. Prostaglandin E2 (PGE2) and F2α (PGF2α) control vascular smooth muscle activity. Prostaglandin I2 (PGI2) is produced by the blood vessel wall and is the most potent natural inhibitor of blood platelet aggregation. Thromboxanes such as TXA2 are produced by thrombocytes (platelets) and are involved in the formation of blood clots and the regulation of blood flow to
the clot. Leukotrienes are made by leukocytes and are extremely potent in causing vasoconstriction and inducing vascular permeability.

**Hormone release**

Steroids are not stored but released immediately to diffuse out of the cell. Protein and peptide hormones are stored in granules within the gland and are released in response to various stimuli. **Trophic hormones** can stimulate hormone release: for example TSH, which stimulates the release of thyroxine. The trophic hormones FSH and LH stimulate the synthesis and release of gonadal steroids, while ACTH stimulates the synthesis and release of adrenal steroids. Hormones can be released in response to nervous stimuli from environmental cues such as light, smell, sound and temperature. This **neuroendocrine transduction** illustrates the integration of the nervous and endocrine systems. Hormones are also released in response to levels of various metabolites. For example, intracellular glucose levels control glucagon and insulin secretion, amino acids stimulate somatostatin release and increase uptake of amino acids, while extracellular Ca2+ regulates parathyroid hormone and calcitonin secreting cells. These effects are all examples of **stimulus–response coupling**.

**Metabolism and excretion of hormones**

Hormones must be metabolized rapidly and removed so that feedback mechanisms can operate and hormones can regulate cellular functions. Removal or inactivation follows exponential decay kinetics. The half-life of the hormone in the circulation is a measure of the longevity of hormone action. Many synthetic hormones and hormone analogues are designed to have a longer half-life and thus be effective for longer periods of time than natural hormones. **Peptide hormones** are degraded by peptidases such as the cathepsins in lysosomes, which split the peptide bonds in the molecule. **Exopeptidases** degrade protein from the amino-terminal or from the carboxy-terminal end. **Endopeptidases**, such as trypsin and chymotrypsin, degrade the protein at internal sites with some specificity. Trypsin hydrolyses peptide bonds where the carboxyl group is from lysine or arginine, while for chymotrypsin the carboxyl group in the peptide bond comes from phenylalanine, tryptophan or tyrosine. Deamination or reduction of disulphide bonds (e.g. insulin) can also inactivate proteins. This occurs in kidney, liver and in target cell lysosomes. **Steroid hormones** are bound to protein carriers in blood, such as serum albumin or steroid-binding globulin, which are necessary since steroids are lipophilic. Binding to protein carriers also increases the half-life of steroids. Physiologically, only 5–10% of the hormone is present in the free or unbound form. Steroids are degraded by a two-phase process in the liver and in the kidneys. This process inactivates the steroids and makes them more water soluble for excretion in the bile. On phase one, enzymes such as cytochrome P450 (CYP) add functional groups such as hydroxyl groups. These metabolites are then conjugated to glucuronic acid or sulphates by transferase enzymes. These more water-soluble metabolites are excreted by the kidney in the urine or by the liver in the bile salts.

**Receptors and Hormone Action**

Hormones interact with receptors located either on the cell surface or inside the cell to initiate their effects on the target tissue. Binding of hormones to cell-surface receptors activates intracellular enzyme systems to alter cell function. Hormones that cross the cell membrane act by binding to intracellular receptors. The hormone–receptor complex then interacts with DNA to affect expression of specific genes. **Extracellular receptors** are large macromolecules located on the outer surface of the plasma membrane in target tissues.

- Hormones regulate specific target tissues.
- How are target tissues “selected” by specific hormones?
Determined through receptors on target cells that provide the specificity for hormone-cell interactions

- Receptors may be components of the cell membrane, cytosolic, or nuclear elements.
- They are a form of a specific binding protein

**Principles of Receptor Binding**

1) **Hormone specificity**
   - Receptors interact with (bind) specific hormones
   - Hormones have a primary receptor, but may interact with less affinity with other receptors
     - Insulin receptor will interact with IGF-1
     - Androgen receptor will bind progestins
     - Glucocorticoid receptor will bind aldosterone
     - Testosterone will bind estrogen and glucocorticoid receptors

2) **High affinity**
   - Receptor affinity is related to concentration of hormone which is the requisite for specificity of receptors. The dissociation constant, $KD$, is the reciprocal of the affinity constant, $KA$, and is usually very low (1nM to 10 pM)

3) **Tissue Specificity**
   - Target tissues containing receptors respond specifically to the ligand (hormone) that binds the receptor. However, presence of a receptor does not necessarily provoke the response of the cell.
   - Normally, an effector system needs to be operational and coupled to the receptor to elicit a response.
   - Some non-specific binding may occur to other tissues, but this binding is of low affinity with no hormonal or tissue specificity.

**Types of Receptors:**

- Single transmembrane spanning receptors without intrinsic tyrosine kinase activity (Prolactin, IGF-I & -II, GH)
- Single transmembrane spanning receptors with tyrosine kinase activity (insulin receptor, PDGF)
- Receptors that span the membrane with 7 units and interact with G-proteins (GnRH, LH, FSH, OXT, TSH)
- Receptors that form ion channels (inositol triphosphate)
- DNA binding receptors (steroid receptors)

**Experimental evidence** that a hormone receptor is located on the cell surface includes:

1. Demonstrating that antibodies against the receptor can block hormone action;
2. Limited proteolysis of intact cells would be expected to destroy the receptor and remove the hormone response;
3. Coupling the hormone to a large molecule that cannot enter the cell and demonstrating that the effect of the hormone is still present;
4. Demonstrating that the receptor is present in a plasma membrane preparation produced by subcellular fractionation (100,000 g pellet).
Receptors can be glycoproteins and contain carbohydrate residues.

**Experimental tools** to demonstrate this are:

1. Treat the receptor preparation with neuraminidase or β-galactosidase to remove the sugar residues. This inhibits binding of the hormone.
2. Concanavalin A (ConA; a protein from jack bean that binds to a D-glucosyl residues) can be used to inhibit hormone binding. In addition, ConA can be used for affinity chromatography of glycoproteins.

**Second messenger systems:**

Some hormones interact with a **cell-surface receptor** and stimulate the synthesis of intracellular **second messenger** compounds. The hormone does not enter the cell to elicit a response but stimulates one of two main pathways:

1. **The adenylate cyclase–cAMP–protein kinase A pathway** or the related guanylate cyclase–cGMP-dependent protein kinase pathway;
2. The calcium-dependent phospholipase C–protein kinase C pathway. In the first system, hormone binding to the receptor activates the enzyme adenylate cyclase or guanylate cyclase, which synthesize either cAMP or cGMP. These second messengers activate protein kinase A. In the second system, binding of the hormone to the receptor activates phospholipase C, which splits phosphatidylinositol in the cell membrane to inositol phosphate and diacylglycerol. The inositol phosphate increases levels of intracellular calcium, which, together with the diacylglycerol, activates protein kinase C. Both protein kinase A and protein kinase C can phosphorylate and activate various intracellular proteins to alter cellular metabolism. These proteins are inactivated by removing the phosphate using the enzyme phosphoprotein phosphatase.

**Intracellular receptors**

Steroid and thyroid hormones operate via intracellular receptors. Receptors for steroid hormones act as transcription factors to regulate the transcription of target genes.

Steroid hormone receptors move between the nucleus and cytoplasm and, in the absence of hormone, are bound to the 90 kDa heat-shock protein complex (Hsp90). (TR, RAR, and VDR do not bind Hsp90.) Binding of the hormone to the receptor results in release of the Hsp90 complex and translocation of the hormone–receptor complex to the nucleus. A dimer of the hormone–receptor complex then interacts with hormone-responsive elements on specific genes to affect DNA transcription. This exposes template sites on DNA, either directly or by influencing pre-existing repressor molecules, to increase the initiation sites for RNA polymerase and increase transcription. These actions of steroid
hormones occur over a much longer period (hours) compared to peptide hormones.

**Pituitary–Hypothalamic Integration of Hormone Action**

The hypothalamus is a part of the brain located below the third ventricle above the median eminence. The pituitary gland or hypophysis is located below the hypothalamus in a hollow pocket of the sphenoid bone known as the ‘sella turcica’ and is linked to the hypothalamus. The pituitary gland consists of two distinct lobes, the posterior pituitary or neurohypophysis and the anterior pituitary or adenohypophysis. The posterior pituitary is nervous tissue that develops as an outgrowth of the diencephalon. It receives hormones that are made in the magnocellular neurones in the hypothalamus and are transported along the axons to the posterior pituitary. The anterior pituitary is glandular tissue, and is subdivided into pars distalis and pars intermedia. It is the ‘master gland’, which produces a number of trophic releasing hormones that stimulate hormone release by target tissues. The hypothalamus is innervated with many neurones from other parts of the body, and receives signals from cells such as baroreceptors and osmoreceptors and other environmental cues. It produces releasing hormones and release inhibiting hormones, which are delivered to the anterior pituitary by the hypothalamic–pituitary portal system. Release from the posterior pituitary is by direct nervous stimulation, from other neurones in the hypothalamus to the neurones that produce the posterior pituitary hormones. The release of hormones from the hypothalamus and pituitary is pulsatile because of the pulsatile firing of nerves. Releasing factors or release inhibiting factors produced by specific neurones in the hypothalamus regulate release of hormones from the anterior pituitary. These factors are produced in very small amounts and are delivered from the hypothalamus to the anterior pituitary by the hypothalamic–hypophysial portal system. Release of hormones by the posterior pituitary is under direct nervous control. The posterior pituitary gland has nerve endings with bulbous knobs that lie on the surfaces of capillaries. Vasopressin or ADH (antidiuretic hormone) and oxytocin are secreted into the capillaries by exocytosis from the neurons that produce them.

**Posterior pituitary hormones**

Oxytocin and vasopressin are the two hormones that are released from the posterior pituitary. Oxytocin causes contraction of smooth muscles. These include the myoepithelial cells for milk let down in the mammary gland, and in the myometrium for the contraction of the uterus for parturition. Vasopressin, also known as antidiuretic hormone, stimulates reabsorption of water from the distal tubular kidney to maintain blood osmolarity when blood volume or blood pressure is decreased. Both vasopressin and oxytocin are polypeptides containing nine amino acids with a disulphide bridge between two cysteines in the molecule. These two hormones are almost identical, except that in vasopressin, phenylalanine and arginine replace isoleucine and leucine of the oxytocin molecule.
They are synthesized as **preprohormones** in the cell bodies of specific neurones in the hypothalamus. The prohormones are cleaved to active hormones during fast axonal transport from the cell body down the axon in the posterior pituitary and before they are released into the circulation. They are released in response to changes in osmotic or barometric pressure, pain, fright or stress, adrenal insufficiency, hypoxia or cardiac failure.

**Anterior pituitary hormones**

The anterior pituitary produces a number of trophic hormones that cause hormone release from target tissues. Hormones of the anterior pituitary are proteins or glycoproteins. They have longer half-lives than releasing hormones produced by the hypothalamus. They are made in specific cells (thyrotrope, gonadotrope, corticotrope, melanotrope, somatotrope, mammotrope) in the anterior pituitary gland.

**Hypothalamic control of pituitary hormone secretion**

The function of the releasing and inhibitory hormones from the hypothalamus is to control the secretion of the anterior pituitary hormones. For each type of anterior pituitary hormone there is usually a corresponding hypothalamic releasing hormone; for some of the anterior pituitary hormones, there is also a corresponding hypothalamic inhibitory factor. The hypothalamic releasing hormones are generally made as a larger prohormone, which is cleaved later to form active peptide hormones. Using labelled antibodies, it can be demonstrated that specific neurons make different releasing hormones. The releasing hormone precursors are made in cell bodies and transported down the axons to the nerve endings for storage. They are released by exocytosis of granule contents (excretion of substances through the cell membrane) into the hypothalamic-pituitary portal blood system in response to electrical signals from other neurones. Chemical signals for release include metabolite levels and other hormones such as steroid hormones or small molecules that can cross the **blood–brain barrier**. The releasing and inhibiting hormones produced by the hypothalamus are all simple peptides or proteins. They range in size from three amino acids for TRH (pyro-E-H-P-amide), ten amino acids for GnRH (pyro-EH-W-S-Y-G-L-R-P-G-amide), 14 amino acids for GH-RIH, 41 amino acids for CRH and 44 amino acids for GHRH. Some hormones (e.g. GH, MSH, PRL) are under tonic inhibition by release inhibiting hormones. These hormones are needed early in life, but as the hypothalamus matures and becomes more active, the hormones that are ‘driven’ by releasing hormones increase, while GH and MSH decrease. Electrical (nervous) stimulus in response to
environmental or internal signals causes the release of releasing hormone by the hypothalamus. The releasing hormone is produced in nanograms and is delivered to the pituitary by the portal system. This causes the release of trophic hormone by the anterior pituitary in microgram amounts. The trophic hormone then causes release of the milligram amounts of the ultimate hormone by the target gland. Note that each step in this process produces an amplification of the response. Hormone secretion from the pituitary gland occurs in an episodic or rhythmic manner. This is regulated by the biological clock in the suprachiasmatic nucleus of the hypothalamus. This may prevent the down regulation of receptors that would occur in response to continuous level of hormone secretion. For example, the levels of cortisol are highest in the morning and decrease in the afternoon and evening. Secretion of somatotrophin is more pulsatile in females than in males. The pulse frequency, pulse amplitude and average hormone levels can be calculated using a pulse detection algorithm. Pulsatile. The nature of this pulsatile release is very important. For example, differences in the frequency of GnRH release by the hypothalamus differentially affect the subsequent release of LH and FSH by the pituitary. The release of hormones by the anterior pituitary is regulated by feedback control. There is a short feedback loop from the anterior pituitary to the hypothalamus and a long feedback loop of the ultimate hormone on the CNS, hypothalamus or anterior pituitary.
In cattle production, good reproductive performance is essential to efficient management and production as a whole, although specific reproductive targets may depend to an extent on local conditions and on individual farm systems and targets. Therefore, it would be helpful to give, at the outset, a brief overview of the various systems of cattle production, their requirements and the importance of reproductive efficiency in attaining them. In some countries, especially in the tropics, much of the cattle production could be described as multi-purpose, with cows being used to provide milk, meat, clothing, fertilizer, fuel, draft power and sometimes for status or as a form of currency. However, for the most part, cattle production may be divided into two sectors: dairy production and beef production. Reproduction is a vital factor in determining the efficiency of animal production. At best, a cow is only likely to produce a single calf per year. Therefore, bovine reproduction is less efficient than in the other farm species, e.g., pigs and sheep. This also means that the rate of genetic progress is likely to be relatively slow.

Reproductive efficiency can be described as a measure of the ability of a cow to become pregnant and produce viable offspring. Infertility or sub-fertility are varying degrees of aberration from typical levels of reproductive performance. Fertility is usually assessed at the economic level by the calving interval, i.e., the period between successive calvings. From a biological point of view, the calving rate is perhaps the most appropriate measure of fertility. This is defined as the number of calves born per 100 services. This also has its drawbacks because it also fails to take account of cows served too long after calving, or never served at all, because of a failure to detect estrus. A 'fertility factor', which is the product of the estrus detection rate and the pregnancy rate (at 45 days post-service). In practice, the estrus detection rate can be estimated from the submission rate – the percentage of cows inseminated within 23 days of the day they were due for service. The pregnancy rate (the percentage of inseminations that result in a positive pregnancy diagnosis) is then calculated and multiplied by the submission rate to obtain a figure for reproductive efficiency, or the 'fertility factor'. Submission rate should be at least 80% and pregnancy rates should be 70%, giving a figure of 56%.

**The calving interval can be divided into two components:**

1. **The calving to conception interval.** This is the time from parturition until the establishment of the next pregnancy. This interval is the main determinant of the calving interval, and is thus the parameter that is usually manipulated in order to try to achieve the target calving interval.

2. **The gestation period.** This is normally between 280 and 285 days in the cow, the variation being mainly due to genetic influences of both the dam and the sire. It can be shortened to only a limited degree by the artificial induction of parturition.

**Factors affecting the calving to conception interval**

In order to achieve a 365-day calving interval the calving to conception interval should not be more than 80–85 days. For the purpose of recording reproductive performance on the farm the calving to conception interval is often subdivided into two components: the calving to first service interval and the first service to conception interval. The calving to first service interval depends on (1) the re-establishment of
ovarian cycles after calving, (2) the occurrence and detection of estrus and (3) the herdsperson’s planned start of services date, if this is later than (1) and (2). The first service to conception interval is dependent on (1) the ability to conceive and maintain pregnancy after a given service and (2) the continuation of ovarian cycles and the correct detection of estrus in those cows that do not conceive to initial services. The interrelationships of the above parameters with blood or milk concentrations of the ovarian hormone progesterone. Calving to first service interval and the first service to conception interval are dependent on both the estrus detection rate and the average conception rate in the herd. Such information can be used to analyse the causes of poor reproductive performance in herds so that appropriate corrective measures can be applied. For example, if a herd’s calving to first service interval is 65 days, the average conception rate is 60% and if the calving to conception interval is 93 days, this indicates that the estrus detection rate is only 50%. Therefore, more attention to the detection of estrus so that 80% of estrous periods are detected would reduce the average calving to conception interval to around 82 days, close to that required for a 365-day calving interval.

In practice, it can be extremely difficult to determine the causes of extended calving intervals. If, for example, the calving to first service interval is extended because estrus was not detected, this could be due to either:

• A failure of the cow to exhibit estrus or
• A failure of the stockperson to detect estrus.

Failure to exhibit estrus might be due to:

• A lack of ovarian activity
• Abnormal ovarian activity or
• A ‘silent ovulation’, i.e., ovulation unaccompanied by estrous behavior.

In addition, if a cow is observed in estrus more than one cycle length (21 days) after service, this could be due to:

• An intervening estrous period having been missed
• Abnormal cycle length
• Loss of a conceptus
• Estrus occurring during pregnancy.

Similarly, if a cow is inseminated at the wrong time (i.e., not within about one day of ovulation), this could be due to either:

• Stockperson error or
• The cow showing estrous symptoms at the wrong time. She may or may not be suffering from an ovarian abnormality, which would interfere with normal fertility.

Thus, the clinician is often faced with a difficult task in trying to unravel the causes of poor reproductive performance.

A fertile cow is one that produces a calf at a regular preferred interval, which will be determined by the management policy for the herd. It must be stressed that a cow must calve at a reasonable time interval to ensure that milk yield does not decline to an unacceptable and uneconomic level.

**The clinical examination**

The clinical examination ideally proceeds through a number of steps. The owner’s complaint, the history of the patient, the history of the farm and the signalment of the patient are usually established at the same time by interview with the owner or keeper of the animal. Observations of the patient and environment are performed next.
Finally a clinical examination of the patient occurs, followed by additional investigations if required.

**Owner’s complaint:** This information usually identifies which individuals and groups of animals are affected. It may also indicate the urgency of the problem. The owner may include the history of the patient and the signalment in the complaint. Stockpersons usually know their animals in detail, and reported subtle changes in behaviour should not be dismissed. However, opinions expressed regarding the etiology should be viewed with caution as these can be misleading. The extent of the problem or the exact nature of the problem may not be appreciated by the owner, and the clinician should attempt to maintain an objective view.

**Signalment of the patient:** Signalment includes the identification number, breed, age, sex, colour and production class of animal. Some diseases are specific to some of these groupings and this knowledge can be useful in reducing the diseases that need to be considered.

**History of the patient(s):** Disease information should include the group(s) affected, the numbers of animal affected (morbidity) and the identities of the animals affected; the number of animals that have died (mortality) should be established. Information regarding the course of the disease should be obtained including the signs observed.

**History of the farm:** The disease history of the farm will indicate diseases that should be considered carefully and may indicate some of the local disease risk factors operating. The sources of information may include farm records, practice records, colleagues and the owner. Husbandry standards, production records, biosecurity protocols, vaccination and estrous synchronization programmes may all be relevant.

**Observation of the environment:** The environment in which the animals were kept at the time of the onset or just before the onset of the illness should be carefully examined. The animals may be housed or outside. Risk factors outdoors may include the presence of toxic material, grazing management, biosecurity and regional mineral deficiencies. Risk factors indoors may include ventilation, humidity, dust, stocking density, temperature, lighting, bedding, water availability, feeding facilities and fitments.

**Observation of the animal at a distance:** Ideally this procedure should be performed with the patient in its normal environment. This enables its behaviour and activities to be monitored without restraint or excitement. These can be compared with those of other members of the group and relative to accepted normal patterns. However, sick animals have often been separated from their group and assembled in collecting yards or holding pens awaiting examination. Observations are most frequently made in this situation; they may include feeding, eating, urinating, defecation, interactions between group members and responses to external stimuli. The patient can be made to rise and walk. The posture, contours and gait can be assessed, and gross clinical abnormalities detected. Useful information is often derived from these observations and this stage in the clinical examination should not be hurried.

**Detailed observations of the animal:** Detailed observations can be made in docile animals without restraint; however, restraint may be necessary to facilitate this procedure. Closer observation of the patient may detect smaller and more subtle abnormalities.

The age of the patient is very important. Maiden heifers have never bred and a small proportion may prove unable to do so. Some may be freemartins being the twin to a male calf and having the genital tract of an intersex. Other congenital defects resulting in infertility are rare but none the less must be considered in such a group of animals. Fertility problems tend to increase with the cow’s parity because the risk of
acquired abnormalities increases with the birth of each calf. Maiden heifers have not yet sustained injuries at calving or experienced problems associated with a retained placenta. These problems are more frequent in older cows. Such animals are more likely to be exposed to dietary deficiencies which can have an adverse effect on fertility. The clinician should seek answers to the following questions:

1. What is the herd size? Has it increased recently?
2. Are fertility records for the herd available?

On many dairy farms retrospective computerized records are kept. A number of indices of fertility may be available and consideration of them all is beyond the scope of this book. Among important indices is the herd submission rate which assesses the vital oestrus detection rate of the herd. The conception rate and its seasonal change monitored by cumulative sum (Q sum) are also very important. Poor fertility indices may indicate overall poor performance. They may also indicate that one section of the herd, for example first calf heifers, is performing badly. It should also be possible to identify how the herd is performing in the current year compared with previous years.

3. Herd management – what staff are employed? Have there been recent changes of staff? What method(s) of oestrus detection are used? Is a fertility control scheme in place? Does it include a postnatal check, prebreeding examination and pregnancy diagnosis? What is the herd policy on cows not achieving herd targets such as being in calf by 82 days?

4. Feeding and production – what feeding regime is used? Has the diet changed recently? Are trace element deficiencies known in the area? Are metabolic profiles taken from cows to assess feeding and identify deficiencies? Does the farm have a seasonal policy for milk production?

5. What is the incidence of herd lameness, metabolic disease and mastitis? Have these problems increased recently?

6. How many cases of abortion occurred during the last year? Was the cause of abortion diagnosed? Is a vaccination policy in place?

7. Is the herd self contained? What was the health profile of any recently purchased animals?

8. Is artificial insemination (AI) used? Are the staff skilled in using AI? Do any staff members have poor cow AI conception rates? Is on-farm semen storage satisfactory? If natural service is used, is the bull known to be fertile?

**History of the cow or heifer**

Further questions should be asked or records inspected to ascertain the following details of the patient’s history:

1. The age and parity of the cow.
2. Has the cow had any previous breeding problems? What were these?
   Was treatment successful?
3. Details of last calving – date, parturient problems including dystocia, retention of fetal membranes.
4. Dates of observed oestrus since calving – has the cow cycled regularly? Is she cycling now? Have her cycles been excessively short or prolonged?
5. Service details – dates and method of service, operator, bull or semen used.
6. Production records of this cow.
7. Health record of this cow – details of lameness, metabolic disease, mastitis, abortion.

**Observation of the patient:**
Cows presented for fertility investigation may be confined to a stall or in the parlour. Wherever possible the cow should be viewed from all sides without restriction, so that her general health and condition can be assessed. Certain specific changes may be seen which relate to the patient’s reproductive state. Many of these are normal physiological changes, but the clinician should look carefully for obvious signs of abnormality which can be investigated further at a later stage. The condition score of the cow should be estimated and confirmed by palpation of the lumbar and sacral regions when the cow is handled. The score (range 1 = very thin to 5 = obese) has an important influence on fertility. Cows should have a condition score of 3 at calving, 2.5 when served and 2.5 to 3 when dried off. The cow in oestrus may appear slightly excitable. A vaginal discharge of clear tacky mucus (the ‘bulling string’) may be present. Scuff marks may be seen on her hindquarters and in front of her tuber coxae caused by the feet of other animals mounting her. Dried saliva from other cows may be seen in similar places. Approximately 48 hours after oestrus the cow may pass a dark red watery vaginal discharge. Animals suffering from long term cyclic ovarian disease may show abnormalities of body shape. Virilism, in which stall-like changes are seen, may occur in animals chronically affected by testes cysts secreting progesterone. Increased development of the neck muscles may occur and the animal may become aggressive. Chronic exposure to oestrogens produced by follicular cysts may produce signs of nymphomania. In addition to displaying frequent signs of oestrus, affected animals may show slackening of the pelvic ligaments with apparent prominence of the tail head. A degree of abdominal distension is anticipated during pregnancy, especially in the last trimester. Animals carrying twins may show greater than normal abdominal distension. Pathological abdominal enlargement may be seen in cases of hydrops allantois or hydrops amnion in which the uterus contains excessive amounts of fluid. The clinical signs of these two conditions are discussed below. Other causes of abdominal enlargement such as ascites must always be borne in mind and should be detected during the general examination. In the last few days of pregnancy the sacrosciatic ligaments become relaxed. The vulva lengthens and appears slightly oedematous. Tail tone appears to be reduced. The udder continues to enlarge and may become oedematous. In some animals the teats leak colostrum. Mucus from the cervical plug may appear at the vulva. Body temperature may fall. Immediately after calving the vulva is still enlarged and a scant bloodstained vaginal discharge is normal for 7 to 10 days. The pelvic ligaments begin to tighten up again and the perineum returns to its preparturient state. A foul brown or red vaginal discharge may indicate the presence of a potentially serious septic metritis. Retained fetal membranes occur quite frequently in cattle. They are usually clearly visible as strands of necrotic chorioallantois and amnion hanging from the vulva. In some animals there is no external sign of retained fetal membranes and these are only detected later during vaginal examination. Prolapse or eversion of the vagina may be seen in the periparturient period. Uterine prolapse with exposure of the endometrium and caruncles may be a complication of the postparturient period. In the normal breeding period, 40 to 82 days after calving, the clinician should look for any signs of abnormal vaginal discharge. At this stage a creamy white discharge, either scant or profuse, may indicate the presence of endometritis.

**Examination of the female genital system:**

This has the following components:

1. **External examination of the female genital system,**
2. **Rectal examination of the genital system including ultrasonographic appraisal,**
3. **Vaginal examination,**
(4) special diagnostic tests if required.
When possible and appropriate all components of the examination should be carried out. At the postnatal check 21 days after calving, specific checks are made for uterine involution, evidence of uterine infection and ovarian activity. When the cow is presented for pregnancy diagnosis and is found to be pregnant further examination is not performed. If not pregnant at pregnancy diagnosis a full gynaecological examination is carried to detect any reasons for failure of conception. For reasons of hygiene, vaginal examination should precede rectal examination. Vaginal examination – especially if a speculum is used – may, however, result in aspiration of air into the vagina which can make subsequent rectal examination more difficult as the vagina feels grossly enlarged. Any air in the vagina can be expelled by gently pressing downwards and backwards on the vagina with the hand in the rectum. If the rectal examination is performed first, care must be taken to ensure that faecal material is not carried into the genital tract.

**External examination of the female genital system:**
Any abnormalities already observed should now be followed up by visual and manual examination with the cow restrained in a crush or Al stall. This examination is chiefly confined to the vulva and caudal vagina. Evidence of a vulval or vaginal discharge may be seen at this stage and will require further investigation. In the last trimester of pregnancy the fetus may be balled through the lower part of the right abdominal wall. Fetal presence and sometimes its viability can be detected.

**Vulva**
The vulval lips should form a seal which, with the cervix, prevents the entry of potentially dangerous organisms into the uterus. The vulva should be positioned vertically below the anus. The lips of the vulva should normally be of approximately similar size and should have no visible space between them.

**Sinking of the anus**
Sinking of the anus in an anterior direction is seen in many older cows. It is also seen in younger animals in certain breeds, for example the Charolais. As a result of anal displacement the upper commissure of the vulva is dragged forwards, causing a variable degree of distortion of the vulva; thus the vulval seal may be compromised and the risk of faecal contamination is increased. At the mucocutaneous junction or just within the vulva areas of necrotic vaginitis may be present caused by fetal or human pressure at calving. Early lesions appear dark and congested; later lesions are green and necrotic. In the immediate postparturient period, the vulval lips may be oedematous and bruising may be present. **Scar formation** in the tissues of the vulval lips may follow injuries sustained during calving. This can also reduce the efficiency of the vulval seal and lessen the likelihood of successful conception.

**Vulval and/or vaginal discharge**
The color, quantity and consistency of this should be observed. Its origin will probably require further investigation. The clear bulling string, the red metoestrus bloodstained discharge and the appearance of retained fetal membranes have been mentioned above. Although some bloodstained or purulent material may originate from the bladder or renal pelvis, most bovine vulval and/or vaginal discharges emanate from the reproductive system. A white or yellowish discharge may indicate the presence of endometritis or pyometra. A foul-smelling bloody and purulent discharge may be associated with acute septic metritis or with a macerated fetus. It
may also occur in cases of abortion or threatened abortion. This type of discharge can also be seen in cases of necrotic vaginitis and in association with infected wounds in the vaginal wall. A black-brown foul-smelling discharge in a very sick animal may be an indication of a clostridial infection in the uterus. The clinician must always check, through a full clinical examination, that any unpleasant odour at the hind end of the patient is actually emanating from the vagina. Granular vulvovaginitis may be caused by mycoplasma infection. Infectious vulvovaginitis, a venereal disease caused by bovine herpes virus I, causes painful inflammation of the external genitalia of the cow and the bull.

**Ballottement**

Ballottement of the right side of the abdomen in the last trimester of pregnancy will often make contact with the fetus and sometimes cause it to move. Signs of movement confirm the presence of fetal life. Absence of movement, either spontaneous or by ballottement, does not necessarily mean that the fetus is dead.

**Rectal examination of the female genital system:**

This must be carried out methodically and with great care and sensitivity. The examination should provide useful information about all palpable parts of the female genital tract. It should reveal the whereabouts, size and condition of the cervix, the uterine body and horns, and the right and left ovaries. It may prove possible to identify and assess the ovarian bursae and the oviducts. These structures may be more readily detected when they are diseased than when they are normal. As a result of rectal examination it should be possible to determine whether the animal is more than 6 weeks pregnant, whether she is cycling and the stage of her estrous cycle.

**Preparing for rectal examination**

The animal must be restrained to ensure animal and operator safety. Confinement in an AI stall or crush is preferable to the milking parlour. Fingernails should be short and all hand jewellery removed before commencing rectal examination. Waterproof protective clothing is required. Both arms should be covered with long plastic sleeves, and ideally these should be changed between cows. Either the right or left hand may be used. Some clinicians prefer to use both hands sequentially during a rectal examination. The fingers and thumb are formed into a cone and the gloved hand is covered with obstetrical lubricant. A further small amount of lubricant may be placed against the anal ring of the patient. The hand is gently but firmly advanced through the anus into the rectum. The anus normally relaxes after a few moments allowing the hand and wrist to enter the rectum. Any faeces are gently removed by enclosing them in the hand and carrying them out through the anal ring. Care must be taken to avoid large quantities of air entering the caudal rectum. The risk of this occurring can be reduced by the clinician easing faecal material through the anal ring without fully withdrawing the hand on each occasion. If distended with air, the rectal wall becomes so tense that palpation of structures such as the uterus becomes quite impossible. The cow can usually be encouraged to expel rectal air. The clinician’s hand is advanced into the anterior rectum where normal peristaltic tension in the walls is still present. By making gentle stroking movements with the fingers on the rectal wall muscular tension is restored and flatus is expelled.

**Ultrasonographic examination of the genital tract:**

This technique has become an increasingly important part of the gynaecological examination of cattle. It provides additional information and also confirmation of the findings at manual examination. A linear array or sector scanner with a probe in the 3.5 to 7.5MHz frequency range is used. The probe, coated with a couplant, is covered with a plastic sleeve before use. Ideally, a separate sleeve or outer sleeve should be
used for each animal. The probe is easily damaged and the clinician should be constantly aware of its vulnerability when using it on the farm. The brightness mode (B mode) scanner produces an image compounded from the reflection of ultrasonic waves directed by the probe into the tissue to be investigated. Water does not reflect ultrasound (it is said to be non-echogenic) and is seen as a black image on the screen. Dense tissue such as bone is impenetrable to ultrasound; it is said to be echogenic and produces a pale grey or white image. Other bodily tissues reflect ultrasound to an extent between the extremes of water and bone. The bovine genital tract is very suitable for ultrasonographic evaluation. The technique enables details of ovarian structure to be demonstrated or confirmed. It is possible to recognise ovarian follicles as non-echogenic structures less than 2 cm in diameter. The waves of follicles that develop during the estrous cycle can be seen and counted at serial examinations. Corpora lutea appear as echodense structures protruding through the ovarian wall. Ultrasound is also very useful in the evaluation of ovarian cysts. The wall thickness of ovarian cysts can be seen and measured. An echogenic band around an ovary may confirm the presence of ovarian bursitis.

Pregnancy diagnosis Ultrasonographic scanning of the uterus and its contents enables pregnancy to be easily and reliably diagnosed at 30 days; this is 12 days before the earliest time that pregnancy can be diagnosed by manual palpation of the uterus per rectum. Twins may also be identified and sexing of fetuses is possible by a skilled operator using a high quality scanner. It is possible that a percentage of very early pregnancies will not survive until term. It is advisable to check by palpation and scan that the animal has maintained pregnancy at 6 to 10 weeks.

Confirmation of fetal life can be demonstrated ultrasonographically from 30 days. It may be possible to see fetal heartbeats and at a slightly later stage fetal movement. Clear non-echogenic amniotic fluid with evidence of fetal viability suggests a healthy pregnancy. Cloudy amniotic fluid with fetal tachycardia or severe bradycardia suggests that fetal life is at risk.

In later pregnancy the fetus or its fluids are clearly demonstrable using ultrasound. The presence of cotyledons involving the uterine wall and the choioallantois can also be clearly demonstrated from 90 days onwards.

The ultrasonographic probe can also be used per vaginam. Very detailed information concerning ovarian morphology can be obtained in this way. The probe is held against the vaginal wall. The ovary is secured per rectum and is carefully brought to the probe for evaluation.

Vaginal examination:
This may be carried out manually or using a speculum. Many cows and most heifers find a manual vaginal examination uncomfortable but tolerate a speculum well. In the absence of a speculum much useful information can be obtained from a careful manual vaginal examination. Two main types of speculum are available. Plastic or polished cardboard tubular speculae with a light source at their distal end allow close and well illuminated examination of the vagina and the cervix. Their tubular parts are interchangeable and sometimes disposable, and a separate tube can be used for each animal. Metal, hinged ‘duck-billed’ specula can also be used. Light can be provided by a pen torch. Before the examination, the perineum and vulva must be carefully washed with warm water and a small amount of dilute antiseptic. The distal end of the speculum is lightly smeared with obstetrical lubricant before being carefully introduced into the vagina. At this point the patient may aspirate air into her vagina, allowing a panoramic view of the vagina and cervix. This is particularly likely to
happen with the duck-billed speculum. The tension on the vaginal walls and cervix may reveal lesions which are not visible in the non-dilated vagina.

**Vaginal walls**
The vaginal walls should be carefully inspected for signs of laceration or superficial damage. Perivaginal haematomata caused by calving injuries may cause distortion of the vaginal wall and reduce the size of the vaginal lumen. Their presence can be readily confirmed by rectal or vaginal ultrasonography when the typical segmented appearance of a haematoma can be confirmed. The external urethral orifice can be seen in the vaginal floor over the pubic bones.

**Vaginal contents**
The small clitoris may be seen on the ventral floor of the caudal vagina. In freemartins the clitoris may be prominent, occasionally surrounded by a small number of long hairs. The vagina is usually severely shortened (<5 cm) in freemartins and there is no cervix. Hymenal remnants are quite rare in cattle. In some cases they occur, often just anterior to the external urethral orifice, as part of the white heifer disease syndrome where there may also be deficiencies in other parts of the tubular structures of the uterus and oviduct. Leiomyoma and squamous cell sarcoma are among tumors occasionally found near the vulval lips or within the vagina. Vertical pillars covered with mucous membrane are occasionally seen in the anterior vaginal and are thought to be embryological remnants. Their presence should be noted, however, as they may cause problems at calving. A double cervix may be seen in some animals.

**Cervix**
The cervix is closely examined. When fully dilated at or immediately after calving the cervix is indistinguishable from the vaginal or uterine walls. It normally closes within a few days of calving, closure being delayed by the presence of infection or retained fetal membranes. The external os of the cervix is visible surrounded by a prominent rosebud-like protrusion into the anterior vagina. In older cows a partial prolapse of the caudal part of the cervical canal into the vagina may be seen. In such cases one or more of the annular rings may be visible. Although noteworthy, this is thought to have no adverse effect on subsequent fertility. Segmental laceration of the cervix may occur at calving and resultant scar tissue may prevent closure of the cervical seal. Such laceration may only be visible when the vagina is dissected with aspirated air. In the non-pregnant cow the cervix is closed but opens slightly when the animal is in oestrus. The vaginal lumen should normally be empty and any contents may be pathological. In cases of urovagina urine runs forward from the external urethral orifice pooling in the anterior vagina where it partially or completely covers the cervix preventing successful conception. The clear thick mucus of the bulling string may be seen through the speculum in animals examined during oestrus. It is much thicker than obstetrical lubricant from which it is easily distinguished. A red watery metoestrous discharge may be seen 48 hours after oestrus. A white or creamy white discharge coming through the cervix and pooling on the vaginal floor is seen in cases of endometritis and pyometra.

**Special diagnostic tests:**
These are only necessary in a few cases of diseases involving the female genital system. They are described in summary form below, together with some of their uses.
**Milk (or plasma) progesterone assay**

This can be used to confirm the presence of an active corpus luteum in one of the ovaries. Elevated progesterone is also seen in luteal ovarian cysts, in cases of a mummified fetus and in pregnant animals 19 to 23 days after service. Falling progesterone levels may be seen in failing pregnancies. Cows correctly diagnosed as being in oestrus have very low progesterone levels. Progesterone can be detected qualitatively or quantitatively in either milk or plasma using an ELISA test. A progesterone level of more than 5 ng per milliliter is regarded as being positive.

**Oestrone sulphate test**

This can be used to confirm the presence of a pregnancy of more than 105 days in cattle. The test is useful in cows in which the pregnant uterus is not accessible to palpation *per rectum* because of adhesions, or if transabdominal ultrasonography is not available.

**Bovine trophoblast protein**

This can be detected in the blood by an ELISA test in early pregnancy.

**Oviduct patency test**

Phenol red dye is placed in the uterus or one of its horns using a Foley catheter. If the oviducts are patent the dye will pass through them into the peritoneum. It passes into the circulation and is excreted through the kidneys. The bladder is catheterized and urine collected at 5 minute intervals. If dye is not seen in the urine within 40 minutes, the oviducts are not patent. An alternative test is the starch grain test in which 1g of sterile granular starch is placed over one ovary using a long needle via the sacrosciatic ligament. The cervical region of the vagina is flushed daily with 5 ml of sterile saline which is then stained with iodine. If the oviduct on that side is patent, starch can be identified in the saline within 48 hours.

**Uterine biopsy**

A sample of endometrium is taken by inserting a long pair of biopsy forceps through the cervix. Histological appraisal may indicate the presence of low grade inflammatory changes within the mucosa.

**How to diagnose a subfertile cow:**

Before performing a clinical examination, it is important to obtain a detailed and accurate history, particularly a breeding history, of the cow. The following should be obtained:

- Age
- Parity (there are certain conditions that can be excluded in nulliparous, as opposed to parous, individuals)
- Date of last calving, together with information on the occurrence of dystocia, retained fetal membranes or puerperal infection
- dates of observed estrus since calving when insemination has not occurred (sometimes referred to as estrus-not-served)
- Presence of any abnormal vulval discharge
- dates of services or inseminations, preferably with the identity of the bull
- If uncontrolled natural service is used, then the date when the bull was first allowed access to the cows
- Previous fertility records, particularly calving– conception intervals and services per conception
- details of feeding, management and milk yield; in suckler cows the number of calves suckled
- details of health, i.e. signs of milk fever, mastitis, ketosis, lameness
- details of fertility of other cows or heifers in the group or herd.
Clinical examination
A good general clinical examination should be undertaken with assessment of body condition score and possibly live weight. The genital system should then be examined in detail; where it is available, transrectal ultrasonography should be used.
- Inspect the vulva, perineum and vestibule for evidence of current or healed lesions and discharges.
- Examine the base of the tail for signs of rub marks, and back and flanks for hoof marks, which might indicate that the individual has been ridden by other cows.
- Explore the vagina by hand or speculum to examine the mucosa and to inspect the mucus.
- Palpate the cervix per rectum to determine its size and position in relation to the pelvic brim, and the uterine horns to determine if involution is complete. Assess the texture of the uterus, the degree of tone, the mobility of the horns and the absence of adhesions. Image the same structures using transrectal ultrasonography. The absence of any signs of pregnancy should be confirmed.
- Palpate the uterine tubes for evidence of in duration or increased size.
- Palpate the ovarian bursa for evidence of adhesions.
- Palpate the ovaries to note their position, mobility and size and to identify the presence of any structures. Confirm the nature of the structures using ultrasonography.

Single blood or milk progesterone assays are useful to identify the presence of luteal tissue if concentrations are high (4–6 ng/ml in plasma or 12–18 ng/ml in milk); sequential assays over several days are better. Specific serological tests – for example, the mucus agglutination or fluorescent antibody tests for Campylobacter fetus, or the investigation of a wide range of infectious agents by taking single or paired blood samples can be diagnostic for many diseases. Swabbing for subsequent bacterial culture and endometrial biopsy are of limited value. The PSP (phenolsulphonphthalein) test for tubal patency can also be used to demonstrate occluded uterine tubes.

The following summary describes a procedure for investigating an infertile animal because of the clinical history, signs and examination, with an indication of a possible diagnosis of the cause and its treatment. These are covered in detail in other lectures.

No observed estrus
Rectal palpation or diagnostic ultrasonography should establish the presence or absence of pregnancy; if the individual is pregnant, it should be recorded. However, if there is any doubt or if it might be, pregnancy at a stage that is too early to be detected by the method used, then a reexamination later is required. If there is no pregnancy, then examination of the ovaries is the next step.

Absence of ovaries
This is uncommon. It is due to ovarian agenesis or freemartinism and hence will be seen only in a nulliparous animal. There is no treatment, and thus the animal should be culled.

Small inactive ovaries
If the ovaries are small, narrow and functionless in a heifer, then this is due to delayed puberty or ovarian hypoplasia. There is no treatment; if delayed puberty is suspected, normal cyclic activity should eventually occur. If the ovaries are flattened, smooth, small and inactive and the horns are flaccid, then this is true anoestrus; confirmation may require a repeat examination or a milk progesterone determination 10 days later. This may be due to high yield, suckling, negative energy balance, intercurrent disease, severe postpartum weight loss or trace element deficiency.

Presence of one or rarely more corpora lutea
There are a number of explanations:
- Pregnancy; if in doubt re-examine later and check records.
- Non-detected estrus; improve detection with increased frequency of observation, heat mount detectors or tail paint, or induce luteolysis with prostaglandin F2α (PGF2α) or an analogue, followed by artificial insemination at observed estrus or at a fixed time.
- Suboestrus or ‘silent heat’; this is most likely at first ovulation after calving. Treat with PGF2α or an analogue as above.
- Persistent corpus luteum; thoroughly palpate the uterus, using retraction forceps if necessary, to confirm the absence of pregnancy. It may be due to pyometra, chronic endometritis, mummified fetus or, rarely, a non-specific cause. Treat with PGF2α or an analogue.

**Small active ovaries**
The identification of follicular activity, perhaps together with a regressing corpus luteum or evidence of recent ovulation associated with good uterine tone, indicates that the animal is coming into estrus, is in estrus or has been in estrus (differentiation between a developing and a regressing CL can be difficult ultrasonographically). Careful inspection of the vulva at the time of palpation may reveal clear mucus, and if there is a small amount of fresh bright red blood then the animal has recently been in estrus (metoestral bleeding). Re-examination in 10 days should reveal the presence of a CL if the cow is undergoing cyclical activity.

**Ovarian cysts (luteal or follicular)**
The presence of one or both enlarged ovaries, containing one or more fluid-filled, thin- or thick-walled structures more than 2.5 cm in diameter, can be confirmed using ultrasonography, and should confirm the diagnosis. A repeat examination several days later will confirm their persistence, and a milk or blood progesterone determination will show the presence of luteal tissue. Treat with PGF2α or an analogue if luteal or, in the case of follicular cysts, with GnRH, human chorionic gonadotrophin (hCG) or progesterone preparations.

**Prolonged interoestrus interval**
The ovaries and genital tract should be examined per rectum. If the ovaries are normal, subfertility may be due to:
- Non-detected estrus; if the interval between successive heats is approximately twice the interoestrus interval, i.e. 36–48 days, then this indicates that one estrus has not been observed or recorded. Irregular intervals that are not the product of the normal interval are likely to be due to incorrect identification of estrus. If large numbers of animals are reported then this suggests that the estrus detection rate is poor. If a susceptible corpus luteum is present, PGF2α can be used to cause luteolysis and estrus in 2–5 days’ time. Methods of improving estrus detection should be implemented.
- Embryonic or fetal death; the interval between successive heats is unlikely to be an approximate multiple of 21, and thus will be some other interval such as 35 or 46 days. In an individual cow, it is probably of no significance, but if a number of animals are involved, especially if natural service is used, specific pathogens should be eliminated and other causes sought.

**Regular return to estrus (Repeat Breeder or cyclic non-breeder)**
The ovaries and genital tract should be examined per rectum to determine the presence of gross abnormalities, such as severe adhesions or uterine infection. This condition can occur only if there is a failure of fertilization or embryonic death before day 12 of the estrous cycle (before or at the time of the maternal recognition of pregnancy). There are a number of possible causes:
• Infertile bull; if a number of cows and heifers are involved he should be examined. If artificial insemination is done by trained inseminators from an approved centre, then poor AI technique can probably be excluded. It must be remembered that there is considerable variation in the fertility of bulls standing at artificial insemination studs, although they should be above a minimal level. Where possible, semen from a bull with a high fertility should be selected. Where DIY AI is performed by the owner or herd manager, then it is important to ensure that the person is adequately trained and that the procedure is being done correctly. In some animals, the cervix can be very difficult to traverse, even by experienced inseminators.

• Incorrect timing of service or artificial insemination; this is unlikely to occur repeatedly, unless the time of ovulation is asynchronous. If a significant number of animals are involved, advice on the correct time may be worthwhile or else fixed-time artificial insemination after the administration of PGF2α or progestogens should be instituted.

• Nutritional deficiency or excess; check diet.

• Occluded uterine tubes; palpate carefully and use the PSP test to confirm.

• Anatomical defects; palpate carefully. If the animal is nulliparous, look for segmental aplasia; if it is a parous animal, check for ovarobursal or uterine adhesions.

• Endometritis; if there are clinical signs, diagnosis is simple but subclinical disease can be diagnosed only by biopsy. If endometritis is suspected, treat with appropriate intrauterine antibiotics, or PGF2α to shorten the luteal phase preceding insemination. If there is evidence of a persistent discharge, the possibility of urine pooling in the anterior vagina should be investigated.

• Delayed ovulation; diagnosis is difficult. Treat with GnRH or hCG at the time of insemination or repeat insemination on the subsequent day.

• Anovulation; diagnosis depends on ovarian palpation or transrectal ultrasonography 7–10 days after estrus to demonstrate failure of ovulation by absence of a corpus luteum. Treat with GnRH or hCG at the time of insemination.

• Luteal deficiency; there is evidence that this is quite common although it is difficult to prove. Once other causes have been eliminated, then a luteotrophic agent, such as hCG, might be worthwhile at 2–3 days after subsequent inseminations to improve corpus luteum formation, or at midcycle to stimulate accessory corpus luteum formation.

**Short interoestrus interval**

This condition is usually identified by other signs of nymphomania and palpation or imaging of ovaries. The cause may be:

• Enlarged ovaries; if either one or, more likely, both contain one or more thin-walled, fluid-filled structures this should confirm the diagnosis of follicular cysts. Treat with GnRH, hCG or a PRID.

• Artificial insemination at the wrong time due to incorrect estrus detection. This is often preceded or followed by an extended interval so that the sum of the two intervals is 36–48 days. If large numbers of cows have the same history, estrus detection should be improved.

**Abortion**

This is defined as the production of one or more calves between 152 and 270 days of gestation; they either are born dead or survive for less than 24 hours. The cow should be isolated, the fetus and fetal membranes should be retained and the case treated as a suspected *Brucella* abortion under the brucellosis scheme. The physical appearance of the fetus and fetal membranes should be noted, the fetus aged approximately and this confirmed by the service or insemination date if available. One endeavours to
eliminate infection as a cause when one is unable to demonstrate organisms in the fetus, fetal membranes, and vaginal and uterine discharges and/or by the demonstration of specific antibodies in body fluids. Where possible the whole fetus should be submitted to the laboratory for cultural examination.

Possible infectious causes of abortion are:

1. **Brucella abortus**; occurs at 6–9 months of gestation.
2. **Leptospira** spp.; occurs at 6–9 months of gestation.
3. **Listeria monocytogenes**; sporadic outbreaks occur at 6–9 months of gestation.
4. **Campylobacter fetus (venerealis)**; occurs at 5–7 months of gestation.
5. **Tritrichomonas fetus**; occurs before 5 months of gestation.
6. **Salmonella** spp., especially *S. dublin*; is usually sporadic with no specific time, although usually about 7 months of gestation.
7. **Arcanobacterium (Actinomyces, Corynebacterium) pyogenes**; is usually sporadic and occurs at any stage.
8. **Myobacterium tuberculosis**; occurs at any stage.
10. **Bacillus licheniformis**; gives rise to sporadic late abortions.
11. **Neospora caninum**; gives rise to late abortions, and is an increasingly diagnosed cause of fetopathy.
12. Infectious bovine rhinotracheitis–infectious pustular vulvovaginitis (IBR–IPV) virus; occurs at 4–7 months of gestation.
13. Bovine viral diarrhoea (BVD) virus; occurs at any stage.

**Sporadic abortions**

1. Perform a statutory brucellosis investigation.
2. Determine if all abortions have been reported and that it is a true sporadic case. If so, proceed to (3); if not, or if there is any doubt, then follow the procedure for an outbreak investigation.
3. Clinical examination of the cow.
4. Examine the placenta for evidence of obvious lesions, particularly fungi or *Bacillus licheniformis*.
5. Submit serum for *Leptospira* serovar *hardjo* serology unless it is a vaccinated herd.
6. Request culture of a vaginal swab for *Salmonella dublin*.
7. Obtain a detailed history of changes in husbandry, movement of livestock, purchase of animals, hiring of bulls, signs of ill-health and age of aborting cows.

**Abortion outbreak**

1. Repeat (1), (2), (3), (4) and (7) above.
2. Ideally, submit one or more fresh whole fetuses and placentas – or several complete fresh cotyledons.
3. Fetal stomach contents (2 ml) should be aseptically collected using a vacutainer or syringe and needle.
4. Collect fluid from thorax or abdomen (2 ml) using the methods described in (3).
5. Submit about 5 g of fresh lung, liver, thymus and salivary gland. All tissues and other samples should be refrigerated and packed with ice, but not frozen.
6. Take air-dried, acetone-fixed impression smears from fresh cotyledons, lung, liver and kidney.
7. Submit formal-saline-fixed cotyledon, fetal liver, heart and lung.
8. Take two 7 ml vacutainers of clotted blood from all cows that have recently aborted.
9. Repeat samples from the same cows as in (8) 2–3 weeks later for possible rising antibody titres in the serum.

EVALUATION OF DAIRY HERD FERTILITY

Regular, accurate evaluation of the fertility status of the dairy herd is an essential part of a control programme. The minimum information required is identity of cow; last calving date; first and subsequent service or insemination dates; confirmation of pregnancy.

Non-return rate to first insemination

This is the percentage of cows or heifers, in a particular group over a specified period, which have not been presented for a repeat insemination within a specific period. The periods are usually 30–60 days or 49 days. This is used, particularly in artificial insemination centers, to monitor the fertility of bulls and the performance of inseminators.

Calving interval and calving index

The calving interval is the interval (in days) between successive calvings; for an individual cow the calving index is the mean calving interval of all the cows in a herd at a specific point in time, calculated retrospectively from their most recent calving date. These two measurements have been used traditionally as a measure of fertility, since they indicate how closely the individual cow or herd approximates to the accepted optimum of 365 days.

Calving to conception interval (CCI)

The calving interval (or index, CI) is the sum of two components, the interval from the last calving ate to the date of conception \(a\) and the length of gestation \(b\). Thus:

\[ CI = a + b \]

Therefore:

\[ CI = 85 \text{ days} + 280 \text{ days} = 365 \text{ days} \]

The calving to conception interval (CCI) is calculated by counting the number of days from calving to the service that resulted in pregnancy (effective service); this is usually the last recorded service date. The CCI is a useful measurement of fertility but requires a positive diagnosis of pregnancy to be made. It is influenced by two factors: how soon after calving the cows are re-bred and how readily they become pregnant when they have been served. The CCI can be expressed thus:

Mean CCI = \(c + d\)

Where \(a\) is the mean calving to first service interval and \(d\) is the mean first service to conception interval. Therefore:

Mean CCI = \(65 \text{ days} + 20 \text{ days} = 85 \text{ days}\)

The mean CCI is a useful measure of fertility, if the interval from calving to first service is stated, since this probably will have the greatest influence upon its length.

Days open

This is defined as the interval, in days, from calving to the subsequent effective service date of those cows that conceive, and from calving to culling or death for those cows that did not conceive. Numerically, it will always be greater than the mean CCI unless all cows that are served conceive, in which case it would be the same. Days open is a popular measurement of fertility in North America.

Calving to first service interval

In the case of a herd that calves all the year round, a mean value of 65 days should result in a mean CCI of 85 days (see above). The factors that influence the calving to first service interval are:

- Breeding policy of the farm. Although cows will return to estrus after calving as early as 2–3 weeks, they should not be served before 45 days, and in the case of first
calvers, high yielding cows and those that have had dystocia and problems during the puerperium slightly longer should elapse. Thus, in a seasonal calving herd, those animals that calve early in the season will have their first service delayed and, for those that calve late, it may be necessary to advance the date of first service, thereby tightening the calving pattern.

- Delayed return of cyclical activity after calving, i.e. acyclicity or true anoestrus.
- Failure to detect estrus in those cows that have resumed normal cyclical activity. Factors (2) and (3) can be improved by ensuring that cows have returned to cyclical activity postpartum. This can be done by regular and routine examination per rectum of those cows that have failed to be seen in estrus by 42 days postpartum and by the use of milk progesterone assays. Detection of estrus depends upon the herd manager knowing the true signs of estrus, having a regular routine, recording the events and using estrus detection aids.

**Overall pregnancy rate**

This (originally called the overall conception rate) is the number of services given to a defined group of cows or heifers, over a specified period, which result in a diagnosed pregnancy not less than 42 days after service. The *first service pregnancy rate* is usually calculated separately and obviously refers to first services only. Thus in a 12-month period, if 100 cows receive 180 services, of which 90 resulted in a confirmed pregnancy, the overall pregnancy rate would be 50%.

**The pregnancy rate is influenced by:**

- the correct timing of artificial insemination, which will be dependent particularly on the accuracy of estrus detection
- correct artificial insemination technique, and handling and storage of semen, especially if ‘DIY AI’ is used.
- good fertility of the bull if natural service is used, and the absence of venereal disease
- adequate nutritional status of cows and heifers at the time of service and afterwards
- complete uterine involution and absence of uterine infection this is especially relevant to first-service conception

The pregnancy rate to first service and overall pregnancy rate are very useful measures of fertility; the latter is used to calculate the *reproductive efficiency* of the herd. The rates for the first service are usually slightly higher than those for all services, because the latter group will include those cows that may be sterile and receive many services before they are culled. Mean values of 60 and 58%, respectively, are obtainable, although in many parts of the world the figures are much lower. In order to identify the influence of management changes, particularly nutrition, it is worthwhile calculating these two parameters on a monthly basis (if there are a minimum of 10 services per month), or expressing them as Cu-Sums.

**Estrus detection**

Improving the detection of estrus has a much greater influence upon reducing the calving to conception interval than improving the pregnancy rates; the latter can only be improved up to a certain level. A number of different methods are used and they all have some measure of error. One method is to determine the number of supposed missed estrous periods. Thus an interval of 36–48 days (2 × 18–24) suggests that one estrus has been missed, and an interval of 54–72 days (3 × 18–24) suggests that two have been missed, although this latter range is fairly wide and can lead to errors. The percentage estrus detection rate (ODR) is calculated thus:

\[
\text{No. of interservice intervals recorded}
\]
ODR = \[
\text{No. of interservice intervals recorded} + \text{No. of missed estrous periods}
\]

Another method is to calculate the mean interservice interval for the herd, so that the ODR is calculated thus:

\[
21 = \frac{\text{Mean interservice interval}}{\text{X 100}}
\]

Poor estrus detection may be due to:
- poor accommodation inhibiting cows from exhibiting overt signs of estrus
- poor lighting or identification of animals
- failure to record signs of approaching estrus and signs of true estrus
- inadequate regimen for observing cows for signs of estrus, perhaps due to the herd manager being overworked. Methods of improving and aiding the detection of estrus.

**Distribution of interoestrus or interservice intervals**

Analysis of the distribution of interoestrus, or more usually interservice, intervals will provide useful information about a number of aspects of the reproductive status and management of the herd. These intervals are subdivided into the following groups:
- (a) 2–17 days, excluding those intervals of 1 day associated with double fixed time artificial insemination;
- (b) 18–24 days, the normal interoestrus interval;
- (c) 25–35 days;
- (d) 36–48 days, twice the normal interoestrus interval; and
- (e) more than 48 days.

In a well-managed herd, with accurate detection of estrus and presentation for service, at least 45% of intervals should be within the 18–24 day range, thus 12% for (a), 53% for (b), 15% for (c), 16% for (d) and 6% for (e). If the percentage for the 36–48-day interval is high and the figures for the 18–24-day interval are low, then this is indicative of poor estrus detection. A large number of intervals in groups (a) and (c) suggests inaccurate identification of estrus, whilst a large number of intervals in groups (c), (d) and (e) could be associated with a late embryonic or early fetal death problem. As with all fertility measurements, they should be evaluated together with other parameters. Using the percentage distribution of the interoestrus and interservice intervals, a single figure referred to as the *estrus detection efficiency* (ODE) is sometimes calculated as follows:

\[
\text{ODE} = \frac{b + d}{a + b + c + 2(d + e)} \times 100
\]

A good ODE would be 50% or more.

**First-service submission rate**

Measurements of estrus detection rates are not very accurate, and for this reason the first-service submission rate can be calculated; this is a measure of how quickly cows are served after they have become eligible for service (after the end of the voluntary waiting period). It is defined as the number of cows or heifers served within a 21- or 24-day period expressed as a percentage of the number of cows or heifers that are at, or beyond, the earliest date at the start of the 21- or 24-day period. Thus once a cow has reached the earliest time after calving that she is ready for service, i.e. above 45 days in all-the-year-round calving herds, then she should be served or inseminated within the next 21 or 24 days. However, pregnancy rates will probably not reach their...
optimum for at least 90 days postpartum. Furthermore, cows that have suffered dystocia or an abnormal puerperium should not be served before 60 days postpartum and should be examined routinely before service. It has been shown that there is a good correlation between the physical state of the uterus, as determined by transrectal examination, and the quantity, colour and smell of mucopurulent discharge and the regeneration of the endometrium. The submission rate is influenced by the time interval to the resumption of normal cyclical activity after calving, the detection of estrus in those cows that have resumed normal cyclical activity, and their presentation for service or artificial insemination. A good submission rate is 80%. A relatively simple method of obtaining a fairly accurate measurement is to list all cows that are ready for service (at or beyond the earliest service date of 45 days, or whatever has been decided upon, since calving) at the start of each 21- or 24-day service period. At the end of this period identify all those that have been served. The percentage submission rate is calculated thus:

\[
\text{No. of cows served that are listed} \times 100
\]

\[
\text{No. of c ws that are listed}
\]

**Reproductive efficiency**

Attempts have been made to calculate a single index that provides an overall measurement of fertility and takes into account many different parameters. One such measurement is the reproductive efficiency (RE) of the herd. It is calculated thus:

\[
\text{RE} = \frac{\text{Submission rate} \times \text{Overall pregnancy rate}}{100}
\]

Thus if the submission rate is high, i.e. 80%, and the overall pregnancy rate is good, i.e. 55%, then the RE is 44. In a herd with a more modest submission rate of 70% and an overall pregnancy rate of 50%, the RE is 35. The advantage of this measurement is that an artificially high submission rate, obtained by an overzealous herd manager presenting cows for artificial insemination when they are not in estrus, will be compensated by a reduced pregnancy rate. Conversely, an overcautious herd manager may have a reduced submission rate but although the pregnancy rate may rise to 65%, producing a reasonable RE value, it is not possible to increase this further.

**Fertility factor**

Another composite measurement can be obtained by calculating the fertility factor (FF). This is obtained following the calculation of the overall pregnancy rate (OPR) and the estimation of the estrus detection rate (ODR). It is calculated thus:

\[
\text{ODR} \times \text{OPR}
\]

\[
\frac{\text{FF}}{100}
\]

Thus if the ODR is 60% and the OPR rate is 50%, then the FF is:

\[
\frac{50 \times 60}{100} = 30
\]

**Fertility index**

Another single index that can be calculated and takes into consideration the pregnancy rate to first service, services per conception, calving to conception interval and culling rate is the fertility index.
Puberty

Puberty may be defined as the time at which estrus first occurs, being accompanied by ovulation. The age of onset of puberty is clearly important since this could possibly prevent an animal’s availability for breeding at the desired time. The earlier that heifers calve, the sooner they can replace cull cows in the milking herd and the quicker will be the rate of genetic gain in the herd (assuming the replacements are superior to the culls). Today the target age at first calving is approximately two years. Ideally a heifer should be gaining weight at a rate of slightly above average around the time of service. Friesian heifers, which are able to calve at around two years, should weigh approximately 330 kg and be increasing in weight by about 0.7 kg/day. Underweight heifers are more likely to conceive if they are on a high plane of nutrition and increasing in weight, whilst those that are overweight should be stable or losing weight. Estrus detection can be a bigger problem in heifers than in cows, partly because they are handled and observed less frequently. Two possible ways of addressing this are (1) the use of a bull and (2) synchronization of estrus. The initiation of puberty is largely a function of the animal’s age and maturity since the female is born with a genetic potential for cyclic reproductive activity. Provided the environmental influences are favourable at this time, then once the ‘biological clock’ is started it will continue for as long as the environment remains favourable. In none of our domestic species is there a physiological change comparable with the menopause of women. Amongst non-seasonal polycyclic animals, such as the cow and sow, 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. When the female 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, a point well illustrated in the gilt, where the mean length of the uterine horns is increased by 58%, the mean weight of the uterus by 72% and the mean weight of the ovaries by 32% between 169 and 186 days of age (Lasley, 1968). Females of domestic species reach the age of puberty at the following times:

- mare: 1–2 years
- cow: 7–18 months
- ewe: 6–15 months
- doe or nanny goat: 4–8 months
- sow: 6–8 months
- bitch: 6–20 months
- queen cat: 7–12 months

The changes that occur at puberty depend directly upon the activity of the ovaries, which have two functions: the production of the female gametes and the synthesis of hormones. Let us consider the changes that occur in the ovary of the young heifer calf. At birth, each ovary may contain up to 150,000 primary or primordial follicles; each consists of an oocyte surrounded by a single layer of epithelial cells, but there are no thecal cells. Soon after birth, the ovaries start to develop and produce growing follicles which consist of an oocyte with two or more layers of granulosa cells and a basement membrane. The stimulus for the development of these follicles is intraovarian, and until the heifer reaches the age of puberty they will develop only to the stage where they have a theca interna and then start to undergo atresia. Further development of these follicles to produce mature Graafian or antral follicles, of which there are about 200 growing follicles at puberty in the heifer, is dependent upon the stimulus of gonadotrophic hormones. Despite the absence of oestrous cycles, there is follicular growth as has been shown using transrectal ultrasonography in calves from 2 weeks of age. It was seen that there were follicular waves in response to folliclestimulating hormone (FSH) secretion that were similar to those of the adult, and that individual follicular development was characterised by growing, static and regressing phases. The onset of puberty is signaled by either the occurrence of the first estrus or the first ovulation; in the ewe lamb these do not occur simultaneously because the first ovulation is not preceded by behavioural estrus. A similar response is seen in sexually mature ewes at the onset of the normal breeding season. The hormone that is primarily responsible for the onset of ovarian activity, and hence puberty, is luteinising hormone (LH). In adult ewes during the normal breeding season, basal LH concentrations increase together with the LH pulse frequency to one per hour during the period of maximum follicular growth. This results in the development of follicles to the preovulatory stage, and their secretion of oestriadiol, which activates the LH surge causing ovulation and corpus luteum formation. In the prepubertal ewe lamb, LH pulses occur at similar amplitudes but much lower frequencies (one every 2–3 hours). As a consequence, follicular growth is insufficient to activate the LH surge necessary for final follicular maturation and ovulation. Experimental evidence in prepubertal ewe lambs has shown that ovarian follicles are capable of responding to exogenous gonadotrophin stimulation, and the pituitary is capable of secreting LH at a frequency to stimulate ovulation. The failure of the prepubertal ewe lamb to undergo ovulation and exhibit estrus is due to the high threshold for the positive-feedback effect of oestriadiol, and thus there is no LH surge.

At puberty, the threshold is lowered, thus allowing the pituitary to respond. This is sometimes referred to as the ‘gonadostat’ theory. Other factors are also involved. The frequency of LH secretion is dependent upon gonadotrophin releasing hormone
(GnRH) from the hypothalamus, which is controlled by an area in the hypothalamus referred to as the neural GnRH pulse generator. Age-related changes in brain morphology and neuronal cytoarchitecture may also be important, since extrapolation from studies performed in rats, for example, has shown an increase in the number of GnRH cells with spine-like processes on the soma and dendrites. In addition, the inhibitory effect of opioid peptides on LH secretion is reduced with age, which may provide a neurochemical explanation for the changes in pituitary sensitivity to oestradiol feedback that occur at puberty. The reason for the ‘silent’ first estrus of the pubertal animal is believed to be because the central nervous system requires to be primed with progesterone before it will respond and the animal will show behavioural signs of heat. The first ovulatory cycle has been shown to be short in pubertal heifers (7.7 ±0.2 days), and the first corpus luteum (CL) not only has a shorter than normal life span but is also smaller in size. One explanation for this is that the dominant follicle, from which the first ovulation arises, had already entered the static phase of growth. The subsequent interovulatory interval was normal. The time of onset of puberty is determined by the individual’s genotype, with smaller breeds of animal tending to be slightly more precocious. However, a number of external factors influence this inherent timing.

Factors affecting timing of puberty

Breed

Dairy breeds appear to reach puberty earlier than beef breeds. The average ages and weights at which heifers of different types reached puberty are shown in Table 4.1 and, larger-type heifers (e.g., Simmental) were younger and heavier at puberty than smaller-type heifers (e.g., Angus). Within a breed and type may affect the age at puberty, it is clear that environmental influences also exert strong effects on prepubertal development.

Nutrition, body weight and live weight gain

It has been known for many years that nutritional status and the rate of live weight gain are important determinants of the time of onset of puberty. In a study of the effect of different planes of nutrition, there was a negative relationship between age at puberty and the rate of liveweight gain, i.e., the faster-growing heifers reached puberty at a younger age. Under average conditions, reproductive cycles would be expected to occur in Holstein/Friesian heifers at an average body weight of 250–270 kg. Heifers fed on very high planes of nutrition may achieve puberty as early as 5–6 months of age although this would be very costly in terms of feed. There is also evidence that the mating of heifers at daily rates of liveweight gains of 1 kg and above may result in poor lactational performance subsequently, due to an inhibition of mammary growth. Under adequate conditions of nutritional management the onset of puberty in heifers is unlikely to be a limiting factor in the achievement of calving at two years of age as commonly practised in many countries. Nutritional influences on the onset of cycling are mediated by hormones such as insulin-like growth factors I and II (IGF-I and IGF-II), insulin and leptin. They are more likely to be limiting in lactating cows and their action.

Season

In those species, which are seasonal breeders, such as the ewe, mare and queen cat, the age at which puberty occurs will be influenced by the effect of season of the year. For instance, a filly born early in the year, i.e. January or February, may have her first estrus in the May or June of the following year, i.e. when she is 16 or 17 months old. A filly foal born late in the year, July or August, may not have her first estrus until she is 21 or 22 months old. The same is true of ewes, which, depending upon the time of
year at which they are born, may reach puberty as early as 6 months or as late as 18 months old. There are many reports that season of birth may influence the onset of puberty in heifers; however, the evidence is often conflicting. A possible explanation of such inconsistencies is that exposure to different seasons during prepubertal development may also have an influence. Photoperiod appears to be a major seasonal factor although temperature, particularly if extreme, may also be involved. Supplemental lighting will reduce the age of attainment of puberty. The stimulatory effects of increased lighting appear to be equally effective at either high or low planes of nutrition.

**Proximity of the male**
Studies in sheep and pigs have shown that exposure to the male of the species will advance the timing of the onset of puberty. This so-called ‘ram or boar effect’ is probably mediated by pheromonal and other sensory cues influencing hypothalamic GnRH secretion.

**Disease**
Any disease which can influence the growth rate, either directly or because of interference with feeding and utilisation of nutrients, will delay the onset of puberty.

**Endocrine changes in prepubertal heifers**
There have been few detailed studies of the hormonal control of the prepubertal period in heifers. It is known, however, that the release of pituitary gonadotrophins follicle stimulating hormone (FSH) and luteinizing hormone (LH) begins shortly after birth. In heifers that first ovulated at around nine months of age, plasma LH and FSH concentrations increased from birth to three months of age and then declined until about six months. The values then increased gradually, peaking again at nine months. The occurrence of LH pulses could be detected at all ages but were of varying frequency. Injected estradiol will result in LH release as early as three months of age. Elevated progesterone levels occurred for 8–12 days before the first estrus. An analogous pattern also occurs in progesterone profiles of some postpartum cows. These transient increases in progesterone in heifers were due to luteal structures embedded within the ovary but not always observable on the ovarian surface. It is not clear whether these luteal structures are a product of a true ovulation. Hypothesis for the control of puberty at least in some species is the so-called gonadostat theory. Under this theory, all components of the endocrine system become potentially functional shortly after birth. LH is released from the anterior pituitary gland and stimulates the production of oestradiol-17b from ovarian follicles. However, the hypothalamo-pituitary unit is excessively sensitive to the negative feedback effect of estradiol and therefore LH secretion is inhibited. Eventually this excessive sensitivity is reduced, allowing gonadotrophin secretion to rise, thereby stimulating follicular development and eventual ovulation.

**Induction of puberty**
Of the various attempts to induce puberty most have included trying to simulate the transient rise in progesterone that occurs prior to the first estrus, by giving either progesterone implants or daily injections usually combined with either an estrogen or pregnant mare serum gonadotrophin (PMSG). The rationale for such treatment is that progesterone suppresses pituitary LH release, stimulating ovarian follicle development. Progesterone is also used to control the time of ovulation in the cyclic cow and to induce ovulation in the non-cyclic cow. However, such treatments have only been consistently successful in animals considered to be already approaching puberty. More recent experiments have been carried out in younger heifers using either repeated injections or slow-release formulations of gonadotrophin releasing
hormone (GnRH), but these also have so far failed to be consistently effective. Exogenous oestradiol-17β to induce the release of an LH surge within 12 hours of administration was successfully used. They postulated that the effectiveness of the induced LH surge in inducing ovulation in prepubertal heifers might depend on the stage of follicular development at the time oestradiol-17β is injected. Regressing follicles or follicles that have not reached dominant status may be unresponsive to the LH surge.

The reason for the 'silent' first estrus of the pubertal animal is believed to be because the central nervous system requires to be primed with progesterone before it will respond and the animal will show behavioral signs of heat. The first ovulatory cycle has been shown to be short in pubertal heifers (7.7 +/- 0.2 days), and the first corpus luteum (CL) not only has a shorter than normal life span but is also smaller. One explanation for this is that the dominant follicle from which the first ovulation arises, had already entered the static phase of growth. The subsequent interovulatory interval was normal.
Terminology

"Estrous" (oestrous in many parts of the world outside North America) refers to the entire cycle; "Estrus" (oestrus) refers to the "heat" stage of that cycle when the female is receptive to the male advances;

The ovarian cycle

Since birth, waves of follicles (primary follicles) will have been developing and migrating to the surface of the ovary. In the absence of the factors required to mature and ovulate them, they cease to grow and begin to regress or degenerate, a process known as atresia. At puberty, the anatomical and hormonal conditions required for regular ovulation are established. The situation is analogous to that at the resumption of ovarian cycles after calving. IGF-I and IGF-II will have stimulated the proliferation of small follicles and insulin will facilitate estradiol production in follicles that could potentially ovulate, whilst the hypothalamic-pituitary axis becomes competent to produce the episodic release of LH necessary for follicle development and oocyte maturation and the LH peak necessary for ovulation. The cow is a polyoestrous animal; therefore, once estrous cycles are established they continue indefinitely unless interrupted by pregnancy. This is in contrast to the ewe, for example, which is seasonally polyoestrous and it undergoes continuous estrous cycles only during certain seasons of the year. In the non-pregnant cow, ovulation occurs at approximately 21-day intervals. A short period before ovulation, the cow normally exhibits ‘estrus behavior’ (sexual receptivity), when she will attract and accept the attentions of a bull. Consequently there is a close relationship between ovarian and behavioral events, ensuring that the female is sexually receptive at the fertile period, i.e., about the time that ovulation takes place. The fact that she is not receptive at other times of the cycle is equally important, ensuring that the bull does not waste his resources when there is no chance of conception. The estrous cycle is classically divided into four phases:

• Estrus, the period of sexual receptivity (day 0)
• Metestrus, the postovulatory period (days 1–4)
• Diestrus (days 5–18) when an active corpus luteum is present
• Pro-estrus (days 18–20), the period just prior to estrus.

The cycle is better described in terms of ovarian function, as consisting of two components, the follicular phase (corresponding to pro-estrus and estrus) and the luteal phase (metoestrus and dioestrus). Behavioural estrus occurs towards the end of
the follicular phase. The cycle is initiated by the release of gonadotrophin releasing hormone (GnRH) from the hypothalamus, which in turn causes the release of FSH from the anterior pituitary gland. This stimulates follicular growth. Of the cohort of primary follicles that has been recruited, and have developed to the antral stage (i.e., those containing a fluid-filled cavity), the most mature (the dominant follicle) responds to rising levels of FSH and becomes destined to ovulate (the pre-ovulatory follicle). The remaining antral follicles cease to grow and they also undergo atresia. This process is to an extent controlled by the production, from the dominant follicle, of inhibin, which acts at the local level in limiting the responsiveness of other follicles, and at the pituitary level by limiting the release of FSH. This mechanism ensures that most cows only ovulate one follicle during each cycle, since there are problems associated with attempting to carry more than one calf to term. About 1% of cows naturally produce twin calves as a result of two ovulations or of the division of the developing embryo to create identical twins. As the pre-ovulatory follicle develops, it continues to increase in size, eventually reaching a diameter of up to 2–2.5 cm. Insulin regulates the production of estrogen by the theca interna and granulosa layers of the follicle. This estrogen has three functions: (1) the initiation of oestrus behaviour, (2) the preparation of the reproductive tract for the processes associated with fertilization, and (3) the initiation of the ovulatory peak of LH.

**Estrous behavior:**

This typically lasts for between 6 and 30 hours with an average of about 7 hours. The duration of estrus is dependent on several factors including age and season of the year and there also appears to be a diurnal pattern in that cows seem to show estrus more frequently at night. The main sign of estrus is that the cow will stand to be mounted by a bull, or by other cows in the herd. During estrus the cow becomes increasingly restless and may bellow frequently. The vulva becomes swollen and the vaginal mucous membrane is deep red in colour. There is often a clear string of mucus hanging from the vulva. Visible oestrous changes are used to indicate the appropriate time for artificial insemination. For the stockperson, the only reliable indication of estrus is that the cow will stand to be mounted by a bull or another cow and can often be seen ‘soliciting’ or apparently encouraging other cows to mount her. A cow in estrus is often described as being ‘in heat’ or ‘bulling’.

**Behavioural changes**
- Aggressiveness.
- Bellowing.
- Restlessness.
- ‘Flehmen lip curl’. This may be displayed by the cow in estrus or, more frequently, a cow that is interested in her.
- Other departures from routine, such as a cow coming into the milking parlour last when she would normally be one of the first, or vice versa. At pasture, she and one or more others particularly interested in her could be the only ones not grazing.

**Physiological changes**
• Increased mucus secretion in the cervix and vagina. This leads to another common and quite reliable sign of estrus: the clear string of mucus that is extruded from the vagina and often adheres to the tail. The cow is often said to have a ‘bulling string’ or to be ‘slimming’. In some cases, such as when cows are continually tied up, this may be the main or only criterion for having them inseminated. However, the timing of expulsion of mucus is variable and insemination could be mistimed by up to two days. If the mucus is cloudy or discoloured, the vagina could well be infected and the cow is not necessarily in estrus.

• At times, especially in cold weather, vapour can be seen rising from the backs of cows in estrus. This results from a rise in body temperature associated either with increased activity or with the physiological changes of estrus.

• In dairy cows there is commonly a drop in milk yield on the day of estrus, which may be due either to reduced production or to an interference with the letdown process. The basic cause could be psychological stress or physiological change. As there are many other causes of reduced milk yield, it is not in itself a very good indication of estrus.

• If the lips of the vulva are parted, they are usually more swollen and a deeper red in colour in an estrous as compared with a non-estrus cow.

• Around two days after the end of estrus, blood or bloody mucus is often seen extruding from the vagina or adhering to the skin around the vulva or on the tail. This results from the increased secretion of blood products (including the white cells that help to combat infection) into the uterine lumen under the influence of estradiol at estrus. Stock people sometimes assume that a cow is not pregnant if she ‘bleeds’, but in fact the occurrence of this metaestrus bleeding is independent of insemination and conception. Increasing estrogen levels from the pre-ovulatory follicle initiate a positive feedback response from the hypothalamus, so that a further GnRH release initiates the pre-ovulatory peak of LH. Meanwhile, episodic releases of LH in a low amplitude, high frequency pattern [intervals of one per hour or more, resulting in higher plasma hormone concentrations, will have contributed to the development of the follicle and the maturation of the oocyte within it. The pre-ovulatory LH surge itself consists of the summation of very rapid pulses of LH secretion. The surge (1) stimulates the process of ovulation, by activating an inflammatory reaction, which thins and ruptures the follicle wall and (2) initiates luteinization of the granulosa and thecal cells of the follicle. The LH surge usually lasts from 7–8 hours and ovulation usually occurs from 24–32 hours after the beginning of the surge. At ovulation, the mature antral follicle ruptures, dispersing its content of follicular fluid in the abdominal cavity, and releasing the unfertilized ovum, still surrounded by cumulus cells. The ovum is collected by the fimbria of the oviduct and transported down the oviduct by a combination of ciliary action and muscular contractions of the oviduct.
wall. Little is known about the mechanism of ovulation, but other reported visual observations of ovarian follicles over the ovulatory period using the technique of laparoscopy. About one hour before ovulation the follicle forms an ‘apex’, the point at which rupture eventually takes place. After ovulation there is a rapid collapse of the follicle wall. As the pre-ovulatory follicle develops, the pre-ovulatory gonadotrophin surge acts on the oocyte, stimulating the resumption of meiosis, which had been suspended early in fetal life at the diplotene stage of the first meiotic division. The resumption of meiosis is characterized by breakdown of the germinal vesicle, chromosome condensation function of the first meiotic spindle, expulsion of the first polar body and arrest in the metaphase of the second meiotic division. These changes constitute oocyte maturation. Expulsion of the first polar body occurs about 8–9 hours after the luteinizing hormone (LH) surge. Two cell divisions then occur quite rapidly, one just before ovulation and the other before fertilization. The first division is a meiotic division in which the chromosomal complement of the oocyte is reduced by half to 30. Instead of four cells being produced by these divisions only one is formed, plus a small ‘polar body’ at each division. These polar bodies are the ‘other half’ of the cell division but are deprived of most of their cytoplasm. The polar bodies invariably degenerate. Thus, as in the male, gamete production involves two meiotic divisions, but by contrast, only one mature haploid gamete results from each primary cell. Ovulation is considered to occur on day 1 of the cycle. However, as with the onset and termination of estrous behavior, the exact timing of ovulation is difficult to establish since continuous observation of the ovaries would be necessary. Most reports therefore have been based on discontinuous observations using techniques such as repeated rectal palpation or observation of the ovaries through a surgical incision in the abdominal wall. Consequently, reported values are quite variable. The literature is, however, unanimous that ovulation occurs some hours after estrus. The average value reported is around 12–15 hours after the end of estrus. The relative timing of the events associated with estrus reported in that study is shown in Table 4.2. There is a very close relationship between the LH surge and ovulation, i.e., 27 hours from the beginning of the surge, and have used this to predict ovulation with considerable accuracy. It must be said here that these studies, now 20–30 years old, have not been repeated in detail in more modern genotypes of cattle. After ovulation, the cells remaining in the ruptured follicle proliferate and form the corpus luteum whose function then dominates the cycle from day 4 to about day 17. One of the functions of the LH surge is to cause differentiation of the follicular cells, including the switch from estradiol to progesterone production. Plasma progesterone
concentrations begin to rise from about day 4 of the cycle, reaching a peak around day 8 and remaining high until day 17. Pulsatile changes in progesterone concentrations occur during the luteal phase and these have been shown to be directly related to pulses of FSH from the anterior pituitary gland. High amplitude, low frequency pulses of LH (approximately one every four hours) occur during the luteal phase, resulting in a low average plasma concentration. Although the LH pulse frequency is relatively low during the luteal phase, it nevertheless appears to be sufficient for the maintenance of luteal function. LH has been considered the main, if not the sole, luteotrophic hormone in the non-pregnant cow. The primary role of high progesterone concentrations during the luteal phase is to prepare the uterus for reception of the embryo that may have resulted from fertilization of the ovum shed at ovulation. Progesterone also exerts a negative feedback inhibition on GnRH and LH release, suppressing further follicle maturation. Nevertheless, waves of follicles continue to grow and regress, leading to transient rises in estrogen levels. During each cycle, most cows undergo either two waves of follicle growth approximately 10 days apart or three waves approximately 7 days apart. It is likely that these wave cycles are more common in maiden heifers. In the non-pregnant cow, the corpus luteum begins to regress after about day 17 – a process known as luteolysis. This is brought about by the influence of uterine prostaglandin F2α (PGF2α), the release of which is initiated by oxytocin produced by the corpus luteum itself. Oxytocin is produced in the corpora lutea of both sheep and cows and plasma concentrations of oxytocin parallel those of progesterone during the estrous cycle of the cow. It is well known that exogenous oxytocin can induce luteolysis in the cow and that it has a physiological role in luteolysis by inducing the release of PGF2α. Specific oxytocin receptors are present on the outer membranes of endometrial cells in the uterus. Binding of luteal oxytocin to the receptor stimulates the conversion of arachidonic acid to PGF2α within the endometrial cell. The concentration of oxytocin receptors is low during the early part of the cycle but increases as the cycle progresses, stimulated by estradiol secretion from waves of follicle growth during the luteal phase. This increase in oxytocin receptor concentration leads to the release of PGF2α from the uterine endometrium via the uterine vein for transport to the ovary. From measurements of the relatively stable PGF2α metabolite, 13,14-dihydro, 15-keto PGF2α (PGFM), it has been shown that PGF2α is released from about day 15 of the cycle in a pulsatile manner and that secretion continues for several days or at least until progesterone concentrations are minimal. The mechanism by which PGF2α causes luteolysis has not been fully established, but the most likely possibility is that it inhibits LH activation of the adenylyl cyclase system in the corpus luteum, preventing the production of progesterone by its large and small cells. By approximately day 17 of the cycle, progesterone concentrations decrease to basal levels, initiating the events leading to the next estrus and ovulation. As the corpus luteum begins to regress 3–4 days before estrus, LH pulse frequency begins to rise. The fall in progesterone and rise in LH result in increasing estradiol concentrations, which eventually ‘triggers’ the LH surge. As luteolysis proceeds, a new pre-ovulatory ovarian follicle begins to mature. If the animal becomes pregnant, the PGF2α release is prevented, and progesterone levels remain high.

During the process of luteolysis, the CLs are invaded by macrophages which produce tumour necrosis factor (TNF); this substance in association with PGF2α probably causes luteolysis. In addition, there is also a suggestion that TNF inhibits estradiol production, thereby removing a luteotrophic source.

**Reproductive tract changes**
Estrogen causes the retention of fluid in the cells making up the vagina, cervix, uterus and fallopian tubes. The whole tract thus feels more turgid on palpation through the rectum. The resultant swelling tends to open up the passage through the cervix, allowing sperm to pass more freely and, fortuitously, facilitating the process of artificial insemination. The swelling also opens up the tubo-uterine junction for the passage of sperm and ova, and ensures that the infundibulum encapsulates the ovary, maximizing the chance that ova pass into the oviduct and are not lost into the body cavity. Estrogen also increases blood flow to the reproductive tract and stimulates the production of mucus by the vagina, promoting the conditions for copulation. In the uterus, estrogen facilitates the passage of larger cells, specifically white blood cells, into the lumen, increasing the resistance of the uterus to any infection, which may be introduced at insemination. These secretions are expelled via the vulva, usually about two days after estrus during so-called Metestrus bleeding.

**Cyclic changes in the vagina:**
The main variations are in the epithelial cells of the anterior vagina and in the secretory function of the cervical glands. During estrus, the anterior vaginal epithelium becomes greatly thickened due to cell division and to the growth of the tall, columnar, mucus-secreting superficial cells. During dioestrus, these cells vary from flat to low columnar. Leucocytic invasion of the vaginal mucosa is maximal 2–5 days after oestrus. Copious secretion of mucus by the cervix and anterior vagina begins a day or so before heat, increases during heat and gradually diminishes to the fourth day after heat. The mucus is transparent and flows readily. Associated with these features of the cervical mucus are variations in its crystallisation patterns, which can be seen when dried smears of mucus are examined microscopically. During oestrus, and for a few days afterwards, the crystals are disposed in a distinct aborisation pattern, while for the remainder of the cycle this pattern is absent. This phenomenon, together with the character and amount of cervical mucus, are dependent on the concentration of estrogen. The postoestrus vaginal mucus shows floccules composed of leucocytes, and, as previously mentioned, blood is frequently present. Hyperaemia of the mucosae of the vagina and cervix is progressive during pro-oestrus and estrus; the vaginal protrusion of the cervix is tumefied and relaxed, so that one or two fingers can be inserted into the cervical os. During metoestrus, there is a rapid reduction in vascularity, and from 3 to 5 days after heat the mucosa is pale and quiescent and the external os is constricted while the mucus becomes scanty, sticky and pale yellow or brown. There are also cyclic variations in vaginal thermal conductivity and vaginal pH, the former rising just before estrus. When pH electrodes were placed in the cervical end of the vagina the pH fell from 7.0 to 6.72 one day before the first behavioral signs of estrus, and at the start of estrus fell again to 6.54.

**Cyclic changes in the uterus:**
During estrus, the uterus is congested, and the endometrium is suffused with edematous fluid; its surface is glistening. The muscularis is physiologically contractile so that when the uterus is palpated per rectum this muscular irritability, coupled with the marked vascularity, conveys a highly characteristic tonic turgidity to the palpating fingers; the horns feel erect and coiled. This tonicity is present the day before and the day after estrus but is at its maximum during heat, and, with experience, the veterinarian can detect estrus on this sign alone. Between 24 and 48 hours after estrus the uterine caruncles show petechial haemorrhages, and these give rise to the postoestrus vaginal discharge of blood. In heifers there are often also associated perimetrial subserous petechiae. During dioestrus the endometrium is covered by a scanty secretion from the uterine glands.
Cyclic changes in the ovaries:
Usually one follicle ovulates and one ovum is liberated after each heat, but twin ovulations occur in 4 or 5% of cows, and triplet ovulations more rarely. In dairy cattle, about 60% of ovulations are from the right ovary, although in beef cattle the functional disparity between the ovaries is not great. The size and contour of the ovaries will depend on the phase of the cycle. It is best to begin by studying the organs of a mature unbred heifer. Post-mortem section of such ovaries will reveal the most significant structures in them to be Graafian follicles and CLs.

Follicular growth and development:
Follicular growth and atresia throughout the cycle is a feature in the cow. Two waves of growth were demonstrated, with the first wave beginning on the third and fourth day, and the second starting on the 12th to 14th day of the cycle. Consequently, a normal follicle of 9–13 mm was present from the fifth to the 11th day before becoming atretic. In the second wave the ovulatory follicle developed, and was 9–13 mm between the 15th and 20th days; the ovulatory follicle is selected at about 3 days before ovulation. Others have observed three waves of follicular development in most oestrous cycles. The most notable feature was the regularity of the number of waves of follicular growth per oestrous cycle, which probably reflected genetic or environmental influences. Follicular growth is under the influence of FSH, with normally one follicle obtaining dominance and subsequently ovulating. The dominance does not appear to be mediated by the effect of inhibin but probably by some yet unknown intra-ovarian mechanism, which does not involve the suppression of FSH secretion. In addition, other metabolic hormones such as insulin growth factor 1 (IGF-1) may also be involved in follicular growth patterns. Thus, during dioestrus several large follicles will be found ranging in size up to 0.7–1.5 cm in diameter. These follicles do not alter the general oval contours of the ovaries but do cause some overall variation in gross ovarian size. The ease of palpating them rectally will depend upon the size, degree of protrusion and relationship with the corpus luteum. During
pro-oestrus and oestrus, the follicle which is soon to rupture enlarges, and ovulation occurs when it has attained a size of at least 1.9 cm. On rectal palpation of the ovaries during heat it is usually possible to detect the ripening follicle as a slightly bulging, smooth soft area on the surface of one of them. Ovulation may occur from any aspect of the ovarian surface, and the shape of that organ subsequently when the CL develops will be chiefly influenced by this site. The point of ovulation is usually in an avascular area of the follicular wall, and consequently haemorrhage is not a feature of bovine ovulation, although there is marked postovulatory congestion around the rupture point, and sometimes a small blood clot is present in the centre of the new CL.

THE MARE:

Dominant follicle becomes the ovulatory follicle. When luteolysis occurs, the LH pulses attain the frequency that is required to progress the dominant follicle to ovulation. FSH induces mitosis and stimulates aromatase activity in granulosa cells and they secrete oestradiol and inhibin: within the follicle, oestradiol binds to receptors (ER) in granulosa cells in which it (1) up-regulates ER and receptors of FSH(FSHR), stimulates aromatase activity, mitosis and secretion of IGF-1: systemically, both oestradiol and inhibin depress FSH secretion by the pituitary gland (negative feedback). (2) Granulosa cells have high number FSHR. (3) The low levels of FSH continue to induce mitosis and to stimulate aromatase activity in granulosa cells (thus producing oestradiol and enlarging the antrum). FSH (4) and oestradiol (1) stimulate granulosa cells to secrete more IGF-1 – which, in turn, (5) enhances the steroidogenic and mitotic actions of FSH and oestradiol on granulosa cells, and enhances the steroidogenic action of LH on theca cells. FSH (6) increases the number of LH receptors (LHR) on granulosa cell. The cell division and the secretory activity accelerate the expansion of the dominant follicle: it may be 16 to 18 mm in diameter at the preovulatory stage. The rapid increase in secretion of estrogen induced by the activities of LH, FSH and IGF-1 leads to a surge of oestrogen (7) which evokes, by positive feedback (8), coincident surges of the gonadotrophins (large surge of LH, smaller surge of FSH) and, consequently, ovulation (9).
The average length of the equine cycle is 20–23 days; the cycles are longer in spring and shortest from June to September. Typically, oestrus lasts 6 days and dioestrus 15 days. Ovulation occurs on the penultimate or last day of heat, and this relationship to the end of heat is fairly constant and irrespective of the duration of the cycle or the length of oestrus; manual rupture of the ripe follicle resulted in termination of oestrous within 24 hours. The diameter of the ripe follicle is 3–7 cm. During the last day 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 postparturient 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. Only fertilised eggs pass into the uterus; non-fertilised eggs remain for months in the uterine tubes, where they slowly disintegrate. All equine ovulations occur from the ovulation fossa; only at the ovarian hilus may occasional protrusions of corpora lutea 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. 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.

During winter anoestrus, both ovaries are typically small and bean-shaped, common dimensions being 6 cm from pole to pole, 4 cm from the hilus to the free border and 3 cm from side to side. Not uncommonly, however, in early spring or late autumn, the anoestrous ovaries are of medium or large size and knobby due to the content of numerous follicles of 1–1.5 cm diameter. 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 post-ovulation 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 estrus 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 dioestrous 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. Anoestrus 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. The endocrinological control of the oestrous cycle is governed by the hypothalamic–pituitary–gonad axis; a similar axis controls the reproductive activity of the stallion. The gonads, in the case of the mare, are the ovaries. Overriding the whole of this control mechanism is the effect of photoperiod; decreasing day length causing oestrous cycles to cease and increasing day length causing them to occur. The plane of nutrition and environmental temperature are also thought to play a part. Day length is perceived by the pineal gland in the base of the brain, which, by means of the hormone melatonin, controls the activity of the hypothalamic–pituitary–ovarian axis. Melatonin is produced nocturnally by the pineal gland and, under the influence of short day lengths, dominates the reproductive system, inhibiting the activity of the axis. As day length increases, inhibition of the axis is removed, allowing gonadotrophin-releasing hormone (GnRH) to be produced by the hypothalamus, so driving luteinizing hormone (LH) and follicle-stimulating hormone (FSH) production by the pituitary. LH is released in a pulsatile manner and the frequency of these pulses is seen to increase from 0.38 to 4.74 pulses day−1 as the mare moves from anoestrus
to the first ovulation of the season. Melatonin is secreted by the pineal gland in two phases: photophase (daytime); and scotophase (night-time). It therefore demonstrates a circadian secretion, with the highest levels of secretion being evident during the scotophase. The presence or absence of daylight is perceived by the pineal gland via neural messages from the retina of the eye. In the absence of light, the conversion of tryptophan to melatonin is driven. The exact means by which melatonin controls the hypothalamus is unclear, but it seems likely to involve dopamine and endogenous opioids, including _endorphin. Prolactin, another major seasonally affected hormone, seems to be responsible in the horse for changes in metabolic rate, increasing the efficiency of food conversion during the winter months, a time of food deprivation. Especially evident in the more native breeds, this demonstrates an innate ability of the equine body to anticipate environmental conditions and respond accordingly. Prolactin is thought, therefore, to translate primarily the changes in day length to seasonal changes in nonreproductive physiology, with only a limited effect of reproductive seasonality. The link or mechanism by which melatonin and prolactin secretions interact is better known in other seasonal breeders than in horses. When day length is appropriate, the hypothalamus is driven to produce GnRH. GnRH release, in common with other reproductive hormones, is tonic and pulsatile in manner. Tonic secretion relates to the background continual level of secretion, whereas pulsatile secretion is superimposed upon this as a series of pulses or episodes of higher levels. Both the level of tonic secretion and the amplitude and frequency of episodes can vary throughout the cycle. An increase in episode amplitude, frequency or tonic secretions causes an increase in average hormone concentrations. Eighty per cent of GnRH released is passed directly down an specialized portal system, the hypothalamic–pituitary portal vessels, to have a direct effect on the anterior pituitary (adenohypophysis), with 20% passing back to the central nervous system to affect behaviour. The level of GnRH in the mare’s circulatory system is, therefore, relatively low, as its passage to the anterior pituitary is directed along these specialized portal vessels. In response to GnRH, the anterior pituitary produces the gonadotrophins, FSH and LH, the target organ for which is the ovaries.

**Inhibin and activin**

It is suggested that the subsequent decline in FSH is brought about, at least in part, by the secretion of inhibin by large follicles as they near ovulation. Inhibin acts as a negative feedback on FSH production by modulating the anterior pituitary response to GnRH, in the form of reducing FSH secretion. Activin has also been isolated in follicular fluid and is reported to have a similar but positive feedback effect, again specifically on FSH secretion.

**Prostaglandin F2a**

PGF2a is difficult to measure in the peripheral circulatory system because of its short half-life and pulsatile manner of release. However, PGF2a has a metabolic breakdown product, prostaglandin F metabolite (PGFM), which has a longer half-life and so is easier to measure in blood serum and plasma. As such, it closely mimics changes in PGF2a. Using levels of PGFM as a guide, it can be seen that PGF2a levels rise between days 14 and 17 postovulation, immediately before progesterone levels start to decline. In mares suffering from retained CL or those that are pregnant, no such rise is detected. PGF2a is known to be secreted by the uterine endometrium and causes luteolysis (destruction) of the CL, thus causing progesterone levels to decline In the mare, PGF2a reaches the ovary via the main circulatory system, not by a local counter-current transport system as seen in the ewe and cow. This can be
demonstrated by hysterectomy, as, in the case of the mare, removal of the uterine horn ipsilateral to (on the same side as) the CL does not result in maintenance of that CL.

**Signs of estrus:**
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, 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’. 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.

**Reproductive behavior**
Estrous behavior of the mare is characterized by increased interest in stallions and proceptive behavior in response to the sexual attractiveness of a stallion. The estrous mare will turn her hindquarters to the stallion and show a characteristic posture with lowered pelvis and straddled hind limbs. This is accompanied by deviation of the tail and exposition of the perineal region, together with “clitoral winking” (rhythmic eversion of the clitoris) and frequently voidance of small quantities of urine. Frequent urination ensures contact to the stallion who will show an olfactory response including frequent flehmen. The behavior of the mare thus may function in chemosensory priming of the stallion for reproduction. In estrous mares, a unique facial expression occurs that is characterized by relaxed facial muscles, ears turned to side and a lowered head. In contrast to estrous mares, luteal phase mares will be less interested in a stallion. If a stallion approaches, they will start to squeal, strike and kick at the stallion.

**Endocrine changes during the estrous 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 15 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 estrus. 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.

**Reproductive activity and cycle length**
Horses are seasonal breeders with ovulatory activity being related to long days. In less domesticated horse breeds, ovulatory estrous cycles occur between May and October. Breeding selection has diminished seasonal reproductive activity as conception early in the year is advantageous to horse breeds performing early in life (“official birth day” 1st January). In consequence, among riding and racing breeds, about 30% of
mares show ovulatory cycles throughout the winter season. The horse is predominately monovulatory. Among less domesticated breeds, double ovulation rate is low, but varies between 7% and 25% in domesticated horse breeds. During the breeding season in spring and summer, average estrous cycle length is about 22 days with 5–7 days of oestrus. Oestrous cycle length is also affected by reproductive stage (e.g. 21.2±1.8 days (±SEM) in lactating and 22.8±1.4 days in non-lactating mares; p < 0.01) and breed. It is approximately 2 days longer in ponies than in horses. Between individual mares, significant differences in estrous length are seen. Transition phases from winter anoestrus to the ovulatory season and back into the anovulatory season coincide with irregular periods of estrous behaviour that may last from several days to weeks and thus vary considerably in length. In fillies, puberty occurs approximately at an age of 12–18 months. This age is again influenced by season. In fillies born very early in the year puberty will not occur before the beginning of the breeding season of the following year. Senescence in old mares is rarely seen and most mares continue to cycle independent of age. However, in old mares, the interovulatory interval may be longer than in young and middle-aged mares due to a slower growth rate of the dominant follicle.

**Endocrine regulation of the estrous cycle**

Hypothalamic function In the horse, pituitary venous blood can be experimentally collected by a direct catheterization technique. This allows determination of GnRH and gonadotrophin release directly from hypothalamic and pituitary outflow. In the mare, the gonadotrophins LH and FSH are considered to be under the control of GnRH alone. So far there is no evidence that a specific FSH releasing factor exists in the horse. In the mare, most hypothalamic GnRH pulses are followed by LH pulses from the pituitary and more than 80% of LH pulses are accompanied by a pulse of FSH. The GnRH pulse frequency changes with the stage of the oestrous cycle and is low during the luteal phase with a GnRH pulse interval of approximately 120 min. On the day of ovulation, the GnRH pulse interval lasts approximately 30 min. This is similar to the GnRH pulse interval observed during the LH surge induced in ovariectomized ewes by oestriadiol treatment. Hypothalamic GnRH release is modulated by steroid feedback mechanisms. However, the existence of steroid receptors on GnRH neurons has not been proven in the horse so far. Therefore, feedback mechanisms have to be mediated by higher brain areas. In the horse, endogenous opioidergic systems are activated by progesterone together with oestradiol. During the luteal phase, opioidergic systems inhibit hypothalamic GnRH and subsequent pituitary LH release while during the follicular phase; they are inactive and allow an increase in LH secretion.

**Pituitary function and gonadotrophin release**

Gonadotroph cells are localized in the pars distalis as well the pars tuberalis of the equine pituitary. Subsets of gonadotrophs that store and produce either LH or FSH (monohormonal gonadotrophs) and bihormonal gonadotrophs that store both gonadotrophins exist together. Whereas in the pars distalis all three types of gonadotrophs have been identified, few if any FSH monohormonal cells exist in the pars tuberalis of the equine pituitary. This heterogeneity in the pattern of LH and FSH storage within the gonadotroph population is considered the morphological basis for the differential regulation of LH and FSH secretion throughout the equine reproductive cycle. The divergent pattern of LH and FSH release is more pronounced in the mare than in many other domesticated animal species. An early periovulatory rise in peripheral concentrations of LH is accompanied by a modest increase in FSH
subsequently declining to its nadir concentration while LH is reaching its maximum. In the mid-luteal phase, a second and robust FSH rise occurs with no concomitant increase in LH. This second FSH surge occurs on different days of the cycle among individual mares. In contrast to other domestic animal species which exhibit a short and pronounced preovulatory LH surge, no distinct periovulatory LH peak exists in the mare. However, during oestrus the period of elevated concentrations of LH lasts for several days. The gradual increase in LH is transiently disrupted after ovulation due to absorption of oestradiol from follicular fluid that is suggested to be discharged into the abdomen at ovulation. Peak concentration of immunological LH activity is reached not earlier than one day after ovulation, but is preceded by biological LH activity. Maximal LH bioactivity occurs shortly before ovulation. In pituitary blood, gonadotrophin pulse frequency rises from 0.5 pulses per hour early in the LH surge to 1.9 pulses per hour at the time of ovulation. In jugular blood, gonadotrophin pulses are relatively small and therefore difficult to detect. This is most likely the result of the long plasma half-life of both equine gonadotrophins (approximately 5 h). During the periovulatory period, the mean frequency of detectable gonadotrophin pulses in peripheral circulation increases to approximately 1 pulse per hour, while during the luteal phase the LH pulse frequency is as low as 0.1 pulses per hour.

**Follicular development, follicular hormone production and ovulation**

Compared to other domestic animal species, the ovary of the mare has a unique structure characterized by an extremely large size and weight (35–120cm3 in volume), the presence of an ovulation fossa and an inversed location of its cortex and medulla. One to two distinct follicular waves develop during an oestrous cycle. A first major follicular wave can occur early in the luteal phase. The dominant follicle of this early wave may be anovulatory, but despite increased concentrations of progesterone ovulation can occur. The development of an ovulatory follicular wave during the luteal phase is a phenomenon considered unique to the equine species. However, it does not occur in all breeds of horses to the same extent: ponies usually develop one major follicle wave, while two follicular waves are typical of thoroughbreds and closely related sport horse breeds (e.g. warmbloods). The emergence of each follicular wave is temporally associated with an FSH surge. FSH reaches a plateau when the largest follicle reaches a size about 13mm in diameter. Subsequently, FSH declines to a concentration that does not support pronounced further growth of subordinate follicles but is sufficient for continuing growth of the dominant follicle. This size dissociation of the members of a follicular wave is known as follicle deviation or selection. Emergence of the future dominant follicle occurs at a size of 6mm in diameter, approximately 6 days before follicle diameter deviation. At the time of deviation the largest and second-largest follicle has an average size of 22 and 19mm, respectively. This phase of the dominant follicle is considered the decisive developmental stage. It coincides with rapid activation of deviation mechanisms that block further development of the second largest follicle to a similar decisive size. The inhibition of growth of the smaller follicles does not depend on follicle-to-follicle inhibitory mechanisms, but follicle deviation involves important changes in the largest follicle. These are characterized by an increased sensitivity to circulating concentrations of FSH. Dramatic changes in the insulin-like growth factor (IGF) system (IGF-I and -II, IGF binding protein, IGF binding protein proteases) in the largest follicle before the beginning of size deviation play a crucial role. Simultaneously, the dominant follicle suppresses circulating concentrations of FSH, most probably due to follicular synthesis and release of oestrogens and inhibin. This
hypothesis is supported by the finding of an increased oestradiol production in the future dominant follicle one day before follicle deviation together with an inverse relationship between circulating concentrations of FSH and inhibin in the cyclic mare. It is feasible that systemic LH is not involved in follicle deviation but is required for further growth of the dominant follicle after the beginning of deviation. With regard to preovulatory follicular development and ovulation horses differ from other farm animal species. The preovulatory follicle is much bigger and ruptures at a specific region of the ovary – the ovulation fossa. From deviation onwards, the preovulatory follicle grows at an average rate of 3mm per day to a diameter of approximately 35mm four days before ovulation. Continued growth occurs up to 2 days before ovulation when follicular size reaches a plateau of approximately 40mm. However, equine follicles may grow up to a preovulatory size of 55mm and more, with mares ovulating consistently from similar preovulatory diameters in consecutive cycles. Histological maturation of the equine preovulatory follicle is characterized by an extensive expansion of its entire mural granulosa cell layer. Furthermore, accumulation of extracellular matrix is abundant. The ovulatory process of the equine follicle involves a specific and unique pattern of gene regulation in theca and mural granulose cells. This includes differences in the expression of a variety of factors among them prostaglandins and prostaglandin metabolizing enzymes. During ovulation, oocyte and corona radiata enter the oviduct, while most of the follicular fluid passes into the peritoneal cavity. Hormones from this fluid are rapidly absorbed into the circulation leading to a pronounced increase in concentrations of inhibin on the day of ovulation. Double ovulations may occur in mares. The double ovulation rate is affected by various factors such as breed, reproductive status and age and pharmacological manipulation of the oestrous cycle. The incidence of spontaneous double ovulation varies between approximately 2% in ponies and 25% in thoroughbreds, respectively. When two dominant follicles (two follicles >28mm) develop in the same follicular wave, double ovulations occur in about 40% of mares. These may occur synchronously (within 12 h), but intervals up to two days and more have been reported between ovulations and can lead to the establishment of a twin pregnancy. During the 2.5 immediately days before ovulation, the rate of dominant follicle growth in double ovulating mares is less pronounced than in single ovulating mares resulting in a lower preovulatory follicle diameter in twin ovulating mares. The reduced follicular growth is related to lower FSH concentrations, most probably due to higher oestradiol concentrations from the two preovulatory follicles.

Luteal function
Due to the specific anatomy of the ovary, the corpus luteum (CL) of the mare enlarges within the internal part of the ovary and does not protrude to the outer ovarian surface as in other species. The equine CL has a pearlike shape and comprises many small compartments with rough surface textures. Structures of the equine CL are formed by luteal and non-luteal cells. As in other species luteal cells can be distinguished into large and small luteal cells. However, in contrast to many other species, luteal cells of the mare are not of thecal origin, but derive exclusively from the granulosa cells of the preovulatory follicle. At ovulation, thecal cells are at various stages of degeneration and are subsequently replaced by hypertrophied fibroblasts. Non-luteal cells of the corpus luteum are mainly fibroblasts, smooth muscle cells, macrophages and endothelial cells that originate from vascular endothelium of the postovulatory follicle. In the early luteal phase, progesterone producing luteal cells show pronounced mitotic activity.
**Luteolysis**

Functional luteolysis in the mare is characterized by a pronounced decrease in blood concentrations of progesterone at around days 15–17 of the cycle. Morphologic regression of the CL develops later and more slowly. Functional luteolysis coincides with a pronounced decrease in expression of P450scc. Simultaneously, the majority of hormone-producing luteal cells undergo cell death characterized by an increase in apoptotic markers. However, during that time a subset of luteal cells still enters the early stages of cell division. Expression of vascular endothelial growth factor (VEGF) declines at early regression of the CL but does not precede apoptosis in luteal cells. Therefore, in the mare functional luteolysis is not initiated by endothelial cell death within the CL and no acute changes in blood flow contribute to luteolysis. A regulatory role of the IGF system not only in follicular but also in luteal function in the mare has been suggested. Most probably, an increase in IGFBP-2 at the time of luteolysis decreases the bioavailability of IGFs in the CL. Thus, functional luteolysis is facilitated by inhibiting the protective effect of IGFs on apoptosis and their stimulatory effect on steroidogenesis. In the mare, the initial signal for luteolysis is endometrial secretion of PGF2_ during the late luteal phase. In contrast to ruminants, PGF2_ is secreted into the peripheral circulation and not into a counter current system (i.e. between uterine veins and ovarian arteries) exists in the horse. However, the equine corpus luteum is believed to have a much higher binding affinity for PGF2_ resulting in a greater sensitivity to this hormone. At day 15 of the cycle, COX-2 expression in uterine epithelial cells of non-pregnant mares is markedly increased. No parallel changes in expression of other enzymes involved in prostaglandin synthesis were found, but COX-2 expression is inhibited in the endometrium of pregnant animals. The regulation of endometrial expression of COX-2 is therefore considered a key event in either induction of luteolysis or maternal recognition of pregnancy in the mare. As in other species, the release of equine endometrial PGF2_ is stimulated by oxytocin. During late diestrus, initial oxytocin secretion is most probably originating from the hypophysis. In contrast to other species, no significant luteal oxytocin synthesis exists in the mare, but the horse is the only domestic species were oxytocin has been localized in the endometrium. Specific secretory endometrial cells containing oxytocin have been described. Therefore, a paracrine–autocrine system involving endometrial oxytocin and PGF2_ release is accelerating luteolysis in the non-pregnant mare.

**Estrous cycle-induced changes of uterus, vagina and endometrium**

During the estrous cycle, the uterus, vagina and endometrium of the mare undergo pronounced changes related to variations in the endocrine milieu. They can easily be differentiated by clinical examination. During estrus, high concentrations of estrogen and low concentrations of progesterone contribute to increasing uterine wall edema together with opening of the cervix and flattening of uterus and vagina. Histologically, the endometrial edema is most apparent in the stratum compactum. It is often associated with accumulation of varying small amounts of fluid within the uterine lumen. Using transrectal ultrasonography the oestrous edema of the endometrium is easily detectable as it results in the appearance of individual endometrial folds. These show areas of different echotexture: nonechogenic areas are corresponding with the oedematous portions of the folds while echogenic areas are corresponding with their tissue-dense central portions. Altogether the endometrial edema results in a characteristic ultrasonographic picture of the uterus of the oestrous mare. In contrast, during the luteal phase the uterine wall has a mild
contractility and the cervix is firmly closed. The uterine echotexture is homogenous and no oedema should be present. In the mare, patterns of secretory and ciliary activity of the endometrium are similar to other species. During oestrus, cells of the polygonal microvillous type dominate the cell population. Secretory cells located around the openings of endometrial glands are of the apocrine or merocrine type. During oestrus, secretory activity of the endometrium together with increased myometrial activity contributes to uterine clearance mechanisms that void uterine infection. Equine endometrial secretory cells produce, store and secrete oxytocin and are thus most likely involved in stimulating and maintaining uterine contractility. Uterine clearance after breeding is further supported by invasive cells, mainly granulocytes and intraepithelial macrophages located particularly in the luminal epithelium of the uterine body. After oestrus, the number of secretory endometrial cells rapidly declines and only few are present during the luteal phase. In parallel, the number of ciliated cells increases and reaches a maximum at mid dioestrus but again declines towards the end of the luteal phase. Endometrial changes related to the oestrous cycle are regulated by the ovarian steroid hormones progesterone and oestrogens interacting with their endometrial receptors. Progesterone and oestrogen receptors exist in the luminal epithelium, glandular epithelium and stroma of the equine endometrium. Their expression is markedly influenced by the stage of the oestrous cycle. It is maximal during oestrus and minimal during late dioestrus suggesting that steroid hormone receptor expression in the equine endometrium is stimulated by oestrogens and down-regulated by progesterone. This is in agreement with the situation in ruminants.

**Follicular dynamic in the mare:**
During early fetal life, primordial germ cells migrate to the primitive gonadal ridge where they proliferate and subsequently enter meiotic division to become arrested in prophase I as primary oocytes. Meiotic arrest continues until atresia or until the primary oocyte is stimulated by an LH surge during post-natal life. Based on histological studies of the developing fetal horse ovary, meiotic divisions begin at about day 70 of pregnancy. Widespread atresia characterizes the first oocytes to enter the meiotic phase between days 73 and 150 of pregnancy with a peak at approximately days 100. Development of oocytes into primordial follicles is not apparent until day 150 and the number of primordial follicles continues to increase during subsequent fetal life so that several thousand of these follicles are contained within the ovaries at birth. Proliferation of the somatic cell layer surrounding the primary oocyte leads to the sequential development of primordial follicles into primary, secondary and, finally, antral follicles, a process that begins in the fetus and continues throughout post-natal life. It is not known how follicles are selected to grow from the pool of primordial, resting follicles in the mare. Adult equine ovaries were reported to contain approximately 36 000 primordial follicles and 100 growing follicles at any one time, with large variability in actual follicle numbers between individual mares. Comparatively higher follicle numbers (threefold) were found in the adult bovine ovary. Proliferative activity of small pre-antral follicles in pony mares was higher at the beginning of the ovulatory season than during anoestrus or at the end of the ovulatory season, whereas follicular atresia was unaffected by season. The equine follicle develops an antrum when it reaches approximately 0.3 mm in diameter. Similar to pre-antral stages, little is known on the development of antral follicles before they reach approximately 2 mm, the smallest diameter that can usually be detected by transrectal ultrasonography. It has been reported that the growth of an equine follicle from 0.1 to 1 mm takes approximately two estrous cycles and that
Atresia during this early phase of antral development is rare. As in other farm species and humans, the development of antral follicles in the horse is characterized by the periodic growth of cohorts of follicles or follicular waves. Characterization of follicular waves has involved the ultrasonic day-to-day identification of individual follicles and the use of a statistical method that avoids the need to maintain the identities of individual follicles during serial ultrasound examinations. Follicular waves in the horse can be identified in relation to follicles 2 mm in diameter and larger; however, it is not known whether earlier antral and pre-antral stages are also characterized by wave-like patterns of growth, a question that has also not been clarified in other species.

**Characteristics of a follicular wave:**

Follicular wave emergence has been normally defined for experimental purposes as occurring when the largest follicle reaches 6 or 13 mm, depending on the study. Identification of wave emergence often requires day-to-day ultrasonic evaluation of the ovary usually after aspiration of all follicles from previous waves. A follicular wave initially involves the simultaneous growth of a variable number of follicles at a common rate of between 2 and 3 mm/day. A recent study involving ablation of all follicles during the middle of an estrous cycle in pony mares reported a mean of approximately 12 follicles emerging during the common growth phase of the ablation-induced wave. Approximately two follicles emerged each day during the first 4 days after wave emergence with a progressive decrease in the numbers of follicles emerging thereafter. The exact number of follicles emerging within waves is affected by factors such as season. The phase of common follicle growth is followed by the selection of a single follicle (occasionally two follicles) which is manifested as a deviation in diameter between the two largest follicles of the wave beginning when the largest follicle reaches approximately 22 mm. Deviation begins a mean of 7 days before ovulation and is characterized by the continuous growth (at an unchanged rate) of the largest (selected) follicle and a dominant follicle and the simultaneous cease in growth and subsequent regression of smaller, subordinate follicles. Once it grows to approximately 35–45 mm, the dominant follicle normally either ovulates or ceases to grow and begins to regress, depending on whether an ovulatory LH surge occurs. The establishment of dominance is mediated by a differential increase in trophic support to the largest follicle of a wave by mechanisms that will be explained in more detail in the next section. This leads to profound changes in follicular cell function that are necessary for the eventual full maturation of the follicle to an ovulatory-competent state and that are reflected in dramatic changes in global gene expression, changes that have begun to be characterized in other species, most notably cattle. Dominance does not seem to be a pre-determined trait among the follicles growing in a wave because follicle ablation studies have demonstrated that all follicles have similar capacity to become dominant, a capacity that in subordinate follicles is lost within approximately 48 h after the beginning of deviation. The same studies showed that in approximately 61% of waves the first follicle to emerge at 6 mm maintains its size advantage over smaller follicles through the common growth phase and becomes dominant. The likelihood of the largest follicle becoming dominant increases as it approaches the expected diameter at the beginning of deviation. In a few instances, yet, the largest follicle ceases or slows down its growth during the common growth phase and is replaced by the second largest follicle (or sometimes even a smaller follicle) which then becomes the dominant follicle.

**Types of follicular waves:**
Follicular waves have been classified as major waves or minor waves, depending on whether they involve the development of a readily identifiable dominant follicle or they produce only smaller follicles, respectively. The number of follicular waves during an estrous cycle varies between species. In the horse, as in humans, only one or two major follicular waves develop during each cycle. A major wave (named primary wave) always emerges during the middle of the equine estrous cycle and produces the ovulatory follicle. Approximately 25% of interovulatory intervals involve an additional major wave (secondary wave) that develops during the first half of the cycle. The incidence of secondary waves is significantly higher in some breeds such as Thoroughbreds, and some of these waves may produce ovulations. The ability to ovulate in the presence of high progesterone levels during dioestrus seems to be unique to the horse. Minor waves (largest follicle usually <30 mm) have been identified at different stages of the estrous cycle and their incidence has been reported to be low, 25% or less of estrous cycles. Yet, a study considering follicles as small as 2 mm concluded that relatively high levels of underlying activity might involve the smallest follicles of a wave.

Emergence of a follicular wave and subsequent selection of a dominant follicle are under finely tuned regulation by systemic mechanisms involving changes in gonadotropin levels and local mechanisms that involve changes in follicular factor levels. Unlike pre-antral follicles, antral follicles cannot develop without adequate gonadotropin stimulation and this has been experimentally shown in the mare. Follicular waves in the mare, as in other species, are temporally preceded by a stimulatory surge in circulating FSH. The acute dependence of follicular waves on FSH has been shown in mares by the inability of follicles to grow beyond a diameter of 15 mm after suppression of circulating FSH by systemic injection of follicular fluid. Close functional relationships between FSH surges and follicular waves involve not only positive effects of FSH on follicles but also negative effects of follicles on FSH. The wave-associated FSH surge reaches a peak when the largest follicle is approximately 13 mm in diameter. The following decline in FSH results from an increase in circulating inhibin, presumably inhibin-A contributed mainly by the three largest follicles of the wave as they grow above 13 mm. The declining FSH concentrations continue to support growth of the follicles of the wave until the largest (future dominant) follicle reaches the expected diameter at the beginning of deviation (approximately 22 mm). At that time, circulating FSH levels become too low to maintain the growth of the follicles of the wave. The low FSH, yet, does not restrict the growth of the future dominant follicle, which by that time has acquired the ability to more efficiently use circulating gonadotropins for growth. The differential responses of the largest and smaller follicles of a wave to gonadotropins result in the initiation of diameter deviation. Continuous suppression of FSH throughout deviation ensures the morphological and functional demise of subordinate follicles and is attributable to production of high levels of inhibin and estradiol by the dominant follicle. The critical role of low FSH levels in the deviation mechanism in mares is illustrated by the disruption of the deviation mechanism after administration of FSH or immunization against inhibin leading to the development of multiple ovulatory follicles. While FSH is particularly important for follicular growth before deviation, LH becomes more critical during deviation. This has been demonstrated by studies showing that experimental suppression of LH in cycling mares leads to the regression of the dominant follicle early during its development, and is consistent with an increase in circulating LH before the beginning of deviation. The study of LH–follicle interrelationships during the anovulatory season has revealed a direct temporal
relationship between an increase in circulating LH, but not FSH, and the development of major waves, further indicating that an increase in LH plays a major role in stimulating the development of dominant follicles. An additional conclusion was that circulating LH concentrations above those required for growth of the dominant follicle are required for the development of ovulatory competence, i.e. for the dominant follicle to become fully responsive to an LH surge. Studies in cattle have shown that LH receptor expression differentially increases in granulosa and theca cells of the early dominant follicle. These findings are consistent with those in other species. Although not critically examined in relation to the beginning of deviation, higher LH receptor levels have been reported in dominant-size follicles than in smaller follicles in the horse. Taken together, the findings on LH levels and LH receptor expression during a follicular wave in mares are consistent with the conclusion that deviation involves a critical increase in the dependence of the dominant follicle on LH. Limited data exist on the involvement of substances other than gonadotropins in the endocrine regulation of antral follicles in mares. Based on observed direct effects on follicle growth or on the presence of specific receptors in equine ovaries, positive roles on follicle growth have been suggested for circulating levels of substances including growth hormone, dopamine and prolactin. Although follicular insulin-like growth factor-1 (IGF-1) in mares largely derives from the systemic circulation, its bioactivity is regulated mostly through local mechanisms and the role of IGF-1 is therefore considered in the next section. A variety of protein and steroid factors including members of the IGF family, estradiol, inhibins and activins, follistatin and vascular endothelial growth factor (VEGF) are involved. In general, these factors act, often in a paracrine manner, to either enhance or diminish the trophic effects of gonadotropins on follicular cells through a variety of mechanisms. Differential changes in the levels of specific factors between follicles thus ensure the continuous development of the dominant follicle and the regression of subordinate follicles during deviation. Differential increase in the levels of estradiol, IGF-1, activin-A and inhibin-A in the future dominant follicle was associated with the beginning of deviation in mares, whereas differentially elevated levels of progesterone and inhibin-B occurred later during the development of the dominant follicle. Studies involving intra-follicular factor injection in mares have provided insight into the complex interrelationships between these factors during follicle deviation. It has been concluded that although all these factors are likely involved in the development of the dominant follicle after the beginning of deviation, only IGF-1 is involved in the initiation of deviation by, among other actions, regulating the levels of other growth factors in the dominant follicle. This is consistent with the particularly important role of the IGF-1 system in follicle selection in other species. Results from a series of in vivo experiments have convincingly confirmed the critical role of the IGF system in follicle selection in mares. Injection of IGF-1 into the second largest or a smaller follicle at the beginning of deviation changed its fate from subordinate to co-dominant resulting in the development of multiple ovulatory follicles. Conversely, injection of IGF binding protein (IGFBP)-3 into the largest follicle at the beginning of deviation resulted in the follicle regressing and being replaced by the second largest follicle which became dominant. At least four types of IGFBPs (IGFBP-2, 3, 4 and 5), which negatively regulate IGF activity, have been identified in equine follicles and the concentrations of three of these (IGFBP-2, 4 and 5) are correlated negatively with those of IGF-1 during follicle development. Consistent with the stimulatory role of IGF-1 in the development of ovulatory follicles, reduced levels of bioactive IGF-1 are thought to be involved in the developmental deficiencies of dominant follicles during
the spring transition that prevent them from acquiring ovulatory competence. An additional factor that has begun to be explored in relation to follicle selection in horses is VEGF. This angiogenic factor has been shown to be necessary for follicle development in other species. VEGF levels differentially increase in the dominant follicle in horses, and this increase is thought to be mediated, at least partly, by IGF-1. VEGF is likely involved in the reported increase in vascularization of the future dominant follicle before the beginning of deviation, which presumably increases the availability of circulating gonadotropins to the follicle. The reduced levels of follicular VEGF and low vascularity of the wall of dominant follicles during the spring transition relative to the ovulatory season underscore the critical role of VEGF in the development of the ovulatory follicle in horses.

Effects of Different Physiological Conditions on Follicle Development in Mares:

1-Effects of season: The effects of season on follicular activity in mares have been studied in considerable detail. Important variations in follicular activity occur not only between the ovulatory and anovulatory seasons but also between different periods within each season. Studies in pony mares using follicle ablation to facilitate the identification of individual follicular waves revealed that, during the ovulatory season, follicular waves periodically occur during the anovulatory season despite the reduced levels of follicle development. Only minor waves (largest follicle <21 mm) occurred during the middle of the anovulatory season, or deep anoestrus, whereas major waves were often detected during the first 2 months (fall transition) and the last 2 months (spring transition) of the anovulatory season, in agreement with previous studies. The onset of the spring transitional period involved an abrupt increase in follicular activity from the low activity typical of deep anoestrus which was reflected in a distinct increase in the diameter of follicles and, most notably, a fourfold increase in the numbers of follicles >12 mm within waves (means of 3.2 and 11.5 follicles in waves developing during deep anoestrus and the early spring transition, respectively). Although dominant follicles during transition may not grow to the diameters typical of ovulatory follicles, in the same study transitional waves produced more follicles than waves developing during the ovulatory season (means of 11.5 and 6.0 follicles >12 mm, respectively) attesting to the high levels of follicular activity even in the absence of ovulation in transitional mares. Based on consistent temporal relationships between follicles and circulating hormones. It has been concluded that the differences in follicle development between different periods of the anovulatory season as well as the deficient development of dominant follicles during the spring transition relative to the ovulatory season are not attributable to deficient circulating FSH levels but rather to changes in LH and, possibly, differences in follicular sensitivity to gonadotropins. Season-related effects on follicular activity have also been reported between the two halves of the ovulatory season, with higher levels of activity during the first half of the season due to higher incidence of both secondary waves and minor waves associated with higher gonadotropin levels.

2-Effects of pregnancy and parturition: Based on combined data from ultrasound and rectal palpation studies it could be concluded that follicular dynamics during the first half of pregnancy are similar to those occurring during the first half of the anovulatory season. The initial period of variable activity (between days 11 and 40 of pregnancy) characterized by the periodic development of major waves or, more commonly, development of sporadic major waves or only minor waves followed by a pronounced decrease in follicular activity in all mares between days 50 and 140 of pregnancy. The diameter of the largest follicle does not exceed 15 mm by day 140. A decrease in follicular activity has also been reported after the first one-third of
pregnancy in cattle. Based on reported hormone–follicle associations, deficient FSH levels do not seem to be responsible for the reduction in follicular growth in some mares between days 11 and 40 of pregnancy an effect that may instead be accounted for by reduced levels of LH (due to persistent progesterone negative feedback), similar to the effects of the seasonal reduction in circulating LH on follicle growth during the fall transitional period. The mechanisms responsible for the dramatic reduction in follicular growth during mid and late pregnancy in mares have not been clarified but likely involve a temporally associated decrease in circulating FSH. This is different from the anovulatory season during which changes in FSH levels do not seem to play a major role in the decrease in follicle growth during deep anoestrus. Reduced follicle numbers during midpregnancy are also likely attributable to ovulation or luteinization of follicles into accessory corpora lutea under the influence of chorionic gonadotropin. Further complexity into the regulation of follicle growth during pregnancy is provided by the observation that the effects of season on hormones and follicles continue to occur during pregnancy. The natural pressure to produce a foal each year in a species with an 11-month-long pregnancy is reflected in an early post-partum ovulation in the mare (foal heat, typically within 2 weeks of parturition). A steady increase in the diameter of the largest follicle and in the numbers of follicles after parturition resulting in ovulation 14 days later was recently shown in Arabian mares. The post-partum increase in follicular growth is induced by an increase in gonadotropin secretion at the time of parturition. Studies have shown that the follicular response to parturition may vary in individual horses or different types of horses, and may not readily occur in primiparous mares, in the presence of a nursing foal or during the winter, when the negative effects of season may prevail over the positive effects of parturition.

3-Follicle development before puberty: Follicle development in spring-born pre-pubertal pony fillies has recently been studied in detail, adding to limited information on follicle profiles from earlier studies. Follicular activity during 2–10 months of life was characterized by a progressive increase in mean follicle diameter (from approximately 6 to 10 mm) and mean follicle numbers (from 3 to 17 follicles). Between 2 and 5 months of age, a short plateau in activity was coinciding with the winter months and re-initiation of follicle growth after 7 months of age leading to the onset of the first ovulatory season in spring. Changes in follicle activity during the first year of life were positively correlated with changes in circulating gonadotropins, consistent with a regulatory role of season on gonadotropin and follicular activity beginning early during life in mares. Follicular growth before puberty was characterized by the development of (minor) follicular waves. Remarkably, these waves were not temporally associated with statistically significant circulating FSH surges, an observation that warrants further investigation.

4-Effects of aging on follicular activity: Many age-related changes in follicular activity in the horse resemble those occurring in humans. Follicular activity during estrous cycles begins to decrease in mares 20 years of age or older eventually leading to a cease in ovarian activity. Intervoluntary intervals first become longer in these mares due to longer follicular phases associated with a primary follicular wave that emerges later and contains less follicles. In addition, the ovulatory LH surge is less pronounced in the older mares, and there is a higher incidence of ultrasonically atypical ovulations characterized by a central hypoechoicgenic area at the ovulatory site. The reduction in follicular activity and frequency of ovulation in mares ≥20 years old is associated with an overall elevation in concentrations of FSH and LH during the follicular phase, a phenomenon that also occurs during the peri-menopausal period in
women. This is eventually associated with persistent ovarian inactivity with follicles <5 mm and no ovulations in the presence of continuously elevated concentrations of FSH and LH. Reportedly, approximately 40% of horses 24 years or older have no ovarian activity whereas 50% of ponies 20 years or older have irregular or absent ovulations. Age-related elevation in FSH concentrations may hasten the eventual depletion of the primordial follicle pool in older mares, similar to what occurs in women. Age-related differences in follicular activity have also been reported in mares during the period of transition into the ovulatory season during which progressively lower follicle diameters and numbers of follicles were found in mares 3–7, 17–19 and >20 years old.

Regulation of seasonal reproductive activity
In the horse, seasonal reproductive activity is stimulated by long days and short nights. In addition to photoperiod, exogenous factors such as age, reproductive state, nutrition, body condition and environmental temperature affect seasonal reproductive activity of the mare tremendously. Therefore, in most horse populations a proportion of mares continue to cycle throughout the year. During the winter anovulatory season which lasts approximately from October to March, prolonged phases of melatonin secretion contribute to reduce hypothalamic GnRH content and release. However, annual reproductive rhythms also occur in pinealectomized horses but are no longer strictly synchronous with the geophysical year. This indicates that seasonal changes in reproductive activity in the horse are driven by an endogenous rhythm that is synchronized to the geophysical year by photoperiod. While mean plasma concentrations of FSH and pituitary content of FSH are relatively constant throughout the year, mean plasma concentrations of LH reach a maximum in summer and are lowest in anovulatory mares. In the peripheral circulation, LH pulses are almost undetectable during winter anoestrus. Pronounced seasonal effects on the incidence of gonadotrophs in the pars tuberalis of the equine pituitary exist. In cyclic animals during the breeding season as well as in sexually active mares during the non-breeding season, the density of gonadotrophs in the pars tuberalis is four to five times higher than in seasonally anovulatory mares. In contrast, no effects of season or reproductive activity on the density of the gonadotrophs in the pars distalis of the pituitary occur. The seasonal pattern in the density of gonadotrophs of the pars distalis is most probably the main mechanism underlying the differential release of LH and FSH throughout the annual reproductive cycle of the mare. In the mare, the anovulatory season can be differentiated into an autumn transitional phase from cyclic activity to deep anoestrus, a mid-anovulatory period and a second transitional phase to cyclic activity in spring. During the autumn transitional phase, inadequate gonadotrophin stimulation in early dioestrus seems to be a critical event leading to suboptimal follicular development and subsequent acyclicity. In the last ovulatory cycle of the season, the periovulatory nadir in concentrations of FSH is prolonged and the early dioestrus FSH surge is reduced when compared to ovulatory cycles during the breeding season. Simultaneously, LH secretion is impaired with a shortened LH surge. Subsequently, the LH surge fails to occur at the expected time of the cycle and results in anovulation. Failure of ovulation is followed by a phase of variable levels of follicular activity before follicular growth is reduced to deep anoestrus levels. During this period, follicular growth is minimal, only a few follicles have a diameter of more than 15mm and the maximal diameter of the largest follicle does not exceed 16mm. A dominant follicle does not develop during that time. Low circulating concentrations of LH contribute to this reduction in follicular growth. However, FSH surges and follicular waves can be distinguished throughout the anovulatory season.
The spring transitional period has a variable length that ranges from 30 to 90 days. Its beginning is characterized by the re-initiation of follicular deviation, i.e. the development of a dominant follicle reaching a size between 20mm and 30mm in diameter. In addition, an increasing number of follicles with a diameter greater than 15mm occur. Subsequently, 1–3 anovulatory follicular waves develop before ovulation occurs. The switch in follicular development between the deep anovulatory phase and spring transition is not attributable to differences in pituitary FSH release. As in sheep, a low expression of follicular FSH receptors during deep anoestrus and a much higher expression in ovulatory mares explain a different sensitivity of follicles to FSH. Most likely, changes at the ovarian level contribute to these differences, among them changes in the expression of dopamine D2 receptors in the ovarian cortex or in the expression of members of the IGF system within ovarian follicles. Seasonal changes in the concentrations of circulating hormones such as prolactin or growth hormone are most likely involved. The most important factor for the re-initiation of ovulatory activity at the end of the transitional phase is the occurrence of surges in circulating LH. As during the ovulatory season, systemic LH is required for further growth of the largest follicle after the beginning of deviation. In contrast to ovulatory follicles, the dominant follicles of the anovulatory follicular waves during spring transition in the presence of lower circulating LH concentrations still show a poorly developed theca, scant vascularisation and a deficiency in steroidogenesis early in the steroidogenic pathway. Resumption of steroidogenesis increases gradually in successive anovulatory follicles during spring transition. A further increase in LH release seems to be the key event that finally leads to the first ovulation.

**Manipulation of reproductive activity in the mare**

Reasons for the manipulation of reproductive activity in the mare are numerous. They range from induction of ovulatory cycles in seasonal anovulatory mares to produce early offspring over timing of ovulation in mares bred with semen of limited availability to suppression of oestrous behavior in performance mares. However, the availability of the drugs used may change and often differs between countries. Moreover, it has to be considered that in brood mares, pharmacological manipulation of the oestrous cycle increases the incidence of twin pregnancies (e.g. 17% in treated vs. 7% in untreated Thoroughbred mares). In luteal phase mares, oestrus is induced by injection of PGF2α and its analogues that will cause immediate regression of the corpus luteum. In comparison to ruminants, small doses of PGF2α are efficient. The treatment is effective from day 5 after ovulation. Onset of oestrus and ovulation occur approximately 3–4 and 8–12 days, respectively, after injection. The interval to ovulation depends on size and stage of ovarian follicles present at drug administration. When a preovulatory follicle at the end of its growing phase is present, ovulation may occur within 48 h after PGF2α treatment. In contrast, in the presence of a small preovulatory follicle or an already atretic follicle, the interval to ovulation is prolonged. For induction of ovulation, oestrus should be confirmed by the presence of a preovulatory follicle (diameter ≥35mm) and uterine oedema. In horses, a single injection of GnRH or its analogues, e.g. buserelin does not predictably result in ovulation making these drugs impractical in the mare. In contrast, either a short-term implant containing the GnRH analogue Deslorelin or human chorionic gonadotrophin (hCG) effectively induce ovulation between 36 and 48 h after treatment (Squires, 2008). Deslorelin may result in receptor down-regulation at the pituitary. If the mare fails to conceive, the following inter-ovulatory interval can thus be prolonged. Therefore, removal of the implant after confirmed ovulation is recommended. Disadvantages of hCG include a diminished response after repeated use due to
formation of neutralizing antibodies and reduced sensitivity to hCG in older mares. Recently, recombinant equine LH has been proven a safe and reliable drug for induction of ovulation. Oestrous can be suppressed either by daily injection of progesterone or oral administration of the synthetic progestin altrenogest. However, ovulation is not always inhibited. Even after long-term treatment with altrenogest, negative side effects neither on fertility nor on general health have been reported. Treatment with altrenogest for one or two days was successfully used to postpone ovulation in oestrous mares without impairing fertility. Exposure of mares to artificial photoperiod is a routine programme for advancing the onset of the breeding season. A 15-h fixed programme starting early in December (in the Northern hemisphere) has been proven to be most effective and advances the onset of ovulatory cycles by 75 days in comparison to untreated mares. As treated mares will still experience a transition period, administration of progestins for a period of 12–15 days as soon as ovarian follicles ≥25mm in diameter develop is common and supports the onset and timing of ovulation. Alternatively, in transitional mares with considerable ovarian activity, ovulation was successfully hastened by twice daily injections of GnRH analogues or eFSH. Experimentally, treatment with the dopamine antagonists sulpiride or domperidone has been used to induce ovulation in transitional phase mares.

THE EWE:
The sexual season of most breeds of sheep in Iraq is from late August-September to November depending on nutrition, the breeding season might start at early June. During which time there are 8–10 recurrent estrous cycles. The stimulus for the annual onset of sexual activity is the declining length of daylight. The extent of the breeding season diminishes with increase of latitude; thus at the equator ewes may breed at any time of year, whereas in regions of high latitude – in both northern and southern hemispheres – the breeding season is restricted and distinct, with a prolonged phase of anoestrustr after parturition. The breed of ewe also influences the duration of the breeding season. Ewe lambs and yearling ewes have shorter breeding seasons than older ewes. The seasonal onset of sexual activity can be advanced by artificial manipulation of the photoperiod and by the use of hormonal agents. The average duration of oestrus in mature ewes of Awassi breed is about 24-30 hours, and is at least 48 hours less in immature ewes. In Merinos, heat may last 48 hours. Ovulation occurs towards the end of oestrustr, and the length of the oestrous cycle averages 17 days.

Reproductive periodicity in sexually mature ewes

Patterns of reproductive activity in the adult, non-pregnant ewe are dominated by two distinct rhythms. The first of these is a 16- to 17-day long estrous cycle. The other is an annual rhythm of ovarian cyclicity characterized by a season-dependent cessation (anoestrus) and restoration (breeding season) of ovulatory ovarian cycles. The latter rhythm, however, is only manifest in ewes derived and managed in temperate climates. The ewe is a seasonally polyoestrous animal with normal ovulatory cycles occurring, in most breeds in the Northern hemisphere, in the fall and winter. The length of the ovine estrous cycle is remarkably consistent throughout the breeding season. There are only small differences, usually not exceeding 1 day, in the duration of the estrous cycle between different breeds of sheep and very little effect of age. Cycles of approximately twice the normal length are occasionally seen in cyclic ewes; it was suggested that such cycles might reflect the incidence of two normal estrous cycles between which behavioral estrus and/or ovulation failed to occur. Recent
ultrasonographic studies have shown that abnormally long cycles in ewes may be associated with a prolonged lifespan of the CL. At the other extreme, short ovarian cycles can be observed in ewes at the beginning of the breeding season and during the post-partum period. These cycles were associated with insufficient luteinization and short-lived CL occurring in sheep at the time of shifts in the pattern of ovarian function from non-ovulatory to ovulatory.

**Stages of ovarian follicular development**

As with many other mammalian species, the development of ovarian germ and somatic cells in sheep progresses through the stages of mitosis and entry into meiosis, followed by initial folliculogenesis and ensuing early follicular development during pre-natal life. Upon reaching the diplotene stage of meiotic prophase, the oocyte is surrounded by a single layer of squamous pre-granulosa cells and a reservoir of primordial follicles is established, numbering from 40,000 to 300,000 in live lambs. Due to uncertain reasons, massive germ cell death involving primordial follicles in the ovaries of sheep occurs by apoptosis from mid-gestation to birth (approximately 20-fold) and further, throughout post-natal life (approximately 55-fold). Primordial follicles continuously leave the non-growing pool of follicles through activation by paracrine factors, and the morphology and proliferation rates of granulosa and theca cells change accordingly. Primary follicles are characterized by a single layer of cuboidal granulosa cells surrounding the oocyte; when ovarian follicles become secondary follicles, they have two or three layers of cuboidal granulosa cells. Theca cells start to differentiate and several fluid-filled cavities form in the follicles which coalesce during the formation of the mature antral (tertiary or Graafian) follicle.

Towards the end of the early stage of folliculogenesis, ovarian follicles become responsive to gonadotrophic hormones, which is a prerequisite to ensuing antral follicular growth and maturation. With the attainment of puberty, the Graafian follicles proceed into the preovulatory stage in which meiosis continues to metaphase II. As the follicular diameter increases to approximately 1–2mm, ovarian ultrasonography becomes a useful tool in tracking the progression of antral follicular kinetics, ovulation, and corpus luteum (CL) formation. It has been estimated that the period of follicular growth from the primordial to the preovulatory stage in ewes exceeds 6 months; the growth from the primordial to the early preantral stage (∼0.2mm in diameter) takes about 130 days; from 24 to 35 more days are required to reach 0.5mm in diameter, and an additional 5 days to reach 2.2mm in diameter; the preovulatory size (at least 4mm in diameter) is typically attained about 4 days later.

**Luteogenesis, mid-cycle luteal function and luteal regression**

The corpus luteum (CL) of the ewe’s oestrous cycle is formed mainly by the action of luteinizing hormone (LH), involving a cascade of functional and phenotypic changes in the granulosa and theca cells of ruptured follicles. LH support is obligatory for initial CL growth and cellular differentiation. Between 3 and 4 days after ovulation, the ovine CL is 6–8mm in diameter and it reaches its maximal diameter of 11–14mm approximately 6 days later. Luteal atrophy in individual ewes occurs abruptly, over the 2–3 days, between days 12 and 15 after ovulation. The CL of the ewe is composed of four major cell types: small luteal cells, large luteal cells, fibroblasts and capillary endothelial cells. Small and large steroidogenic luteal cells derive from the theca and granulosa cells, respectively. However, treatment of ewes with LH may promote transformation of small luteal cells into large luteal cells. Both small and large luteal cells contribute to the increment in the total luteal mass during the early and mid-luteal phase of the oestrous cycle. Large luteal cells increase in size between days 4 and 12 of the cycle (day 0 = onset of oestrus) but their number remains relatively
constant until the onset of luteolysis. In contrast, the size of small luteal cells does not change but, due to mitotic divisions, there is an increase in the number of these cells from days 4 to 8 of the cycle. The capillary endothelial cells and luteal fibroblasts increase in number between days 4 and 12, and between days 8 and 16 of the cycle, respectively. It was also suggested that under certain conditions fibroblasts might differentiate into small luteal cells, which would explain the existence of luteal cells in ewes that are intermediate in their microscopic morphology between fibroblasts and small luteal cells in the luteal glands of sheep. Prostaglandin F2α (PGF2α) secreted by uterine endometrial glands is the luteolytic factor in ruminants. PGF2α is transported to the ovary through the local passage from the uterine venous and lymphatic vessels to the ovarian artery. Towards the end of the luteal phase, follicular oestradiol increases the secretion of PGF2α and promotes the formation of endometrial receptors for oxytocin; this is significantly enhanced by previous exposure to progesterone. The decline in circulating concentrations of progesterone at the onset of functional luteolysis is associated with the occurrence of another increase in oxytocin receptor levels and pulsatile secretion of PGF2α. The increase in endometrial oxytocin receptor level can be detected as early as 1 week after progesterone withdrawal in ewes. Luteal oxytocin and possibly oxytocin of the posterior pituitary origin play an important role in the mechanism that controls PGF2α secretion in sheep.

**Hormonal profiles during the ewe’s estrous cycle**

The oestrous cycle is associated with a sequence of interrelated endocrine events regulated by the hypothalamus producing GnRH, the pituitary gland secreting FSH, luteinizing hormone (LH) and oxytocin; ovarian antral follicles secreting oestrogens and inhibin; the CL secreting progesterone and oxytocin; and the uterine endometrium producing PGF2α. Ovarian follicle development and maturation, steroidogenesis, ovulation, and the formation of CL are primarily controlled by the pituitary gonadotrophins. The regulation of the secretion and bioavailability of gonadotrophic hormones depends on a complex interaction between several internal and external factors. The internal factors in question include locally produced amino acids and peptide/protein hormones, ovarian steroids and other follicular hormones such as inhibin, activin and follistatin, neurotransmitters and neromodulators, and uterine products. The external factors including photoperiodic signals, male pheromones, nutrition and stress, are also known to affect the function of the hypothalamo-pituitary-ovarian axis. Regulation can be achieved directly, through effects on GnRH secretion, or indirectly, by altering pituitary responsiveness to GnRH or ovarian sensitivity to gonadotrophins, heterogeneity of LH/FSH (e.g., hormone glycosylation), local blood flow, or the counter-current exchange of hormones between lymphatic and blood vessels. 3.1. Gonadotrophic hormone secretion The preovulatory discharge of GnRH, and subsequently of LH and FSH, reaches a peak about 14 h before ovulation. This gonadotrophin surge is evoked and sustained by decreased progesterone and increased oestradiol secretion during the final stage of the oestrous cycle. Rhythmic LH pulses generated by GnRH prevail at all reproductive states in ewes, including the period before, during and after the preovulatory surge of gonadotrophins. An increase in LH pulse frequency and amplitude heralds the preovulatory LH surge. The amplitude of LH increases appears to be greater on the down-slope than on the up-slope of the surge. An increment in basal (non-pulsatile) LH release during the surge has also been suggested. Progesterone and oestradiol acting in concert modulate the frequency and amplitude of LH pulses and pulsatile release of LH is inversely related to circulating levels of luteal progesterone. During
metoestrus and early dioestrus, basal serum LH concentrations and LH pulse frequency gradually decline, whereas LH pulse duration and FSH pulse frequency increase. LH pulse amplitude increases at the end of the growth phase of the largest ovarian follicles early in the luteal phase, and the amplitude and duration of LH pulses increases 1 day after the rise in serum progesterone concentrations above the basal levels. Mean and basal serum FSH concentrations increase at the time of follicle wave emergence. Interestingly, FSH pulse frequency increases during the growth phase of large antral follicles developing during early dioestrus, and the formation of the CL is associated with a transient decline in mean and basal FSH levels and FSH pulse frequency. Mean and basal LH concentrations (i.e., LH concentrations after the removal of pulses), and LH pulse frequency increase with decreasing progesterone concentration at the end of the cycle, and mean and basal FSH concentrations reach a peak on the day of follicular wave emergence before declining to a nadir by 2 days after emergence of the last follicular wave of the interovulatory period. None of the parameters of pulsatile LH secretion vary with either the emergence of the final follicular wave or with the end of the growth phase of the largest follicle of the penultimate wave of the cycle. These observations indicate that LH secretion during the luteal phase of the ovine oestrous cycle reflects primarily the stage of the development and secretory activity of the CL, and only a rise in LH pulse amplitude may be linked to the growth phase of follicles emerging early in the cycle. Increases in mean and basal FSH concentrations are tightly coupled with the days of follicular wave emergence throughout the ewe’s oestrous cycle, and FSH pulse frequency increases during the follicle growth phase at mid cycle but declines at the onset of increased luteal progesterone secretion. Regular fluctuations in daily serum concentrations of FSH, with a periodicity of approximately 4 days, occur throughout the ovulatory cycle and season of anoestrous sheep, and they can also be detected in ovariectomized ewes. The major characteristic of the pattern of FSH secretion during the periovulatory period is the occurrence of two consecutive surges. The first of these surges is coincident with the preovulatory LH surge, and the second occurs between 20 and 36 h afterwards. The secondary FSH discharge is lower in amplitude but of a longer duration (20–24 h) compared to the preovulatory surge (11–12 h). The preovulatory FSH surge is preceded by relatively low levels of FSH, while serum LH, oestradiol, and inhibin concentrations are increasing. This suggests that the preovulatory FSH surge arises from the action of GnRH that overrides inhibitory effects on FSH release during that time. The second FSH surge occurs after ovulation, which effectively terminates the secretion of follicular FSH inhibitors.

THE DOE (NANNY) GOAT:

The breeding season in Britain is from August to February with the greatest activity in the months of October, November and December. Near the equator, there is no evidence of seasonality but continuous cyclic activity. The doe is polyoestrus, with an interoestrus interval of 20–21 days, although it is rather irregular at the beginning of the breeding season. The duration of oestrus is 30–40 hours, with ovulation occurring 12–36 hours after the onset. The detection of heat in a doe is difficult in the absence of a male goat. The vulva shows some evidence of oedema and hyperaemia; the tail is wagged from side to side and up and down (the most reliable sign). The doe is restless and more vocal, has a reduced appetite and milk yield, and shows mounting behaviour. The presence of the pheromones from the male goat, which can be transferred from the scent gland on to a cloth, will often intensify the signs.
Goats show a seasonal pattern in reproductive activity related to the annual variations of photoperiod. Onset and length of their breeding period throughout the year is dependent on different environmental and physiological factors (latitude and climate, food availability, breed and breeding system). The fact that their sexual activity is seasonal affects the distribution of their production over the year and this is a problem both in dairy and meat production systems which attempt to have a constant production year-round.

**Estrous cycle**

The oestrous cycle consists of all morphological and physiological changes in the ovaries and genital tract leading to oestrus expression (phase of receptivity towards males) and ovulation and the preparation of the genital tract for copulation, fertilization and embryo implantation. During the course of the breeding season, females can undergo several oestrous cycles successively and the number of successive cycles is dependent on the length of the breeding season and the breed of goat. The length of oestrous cycle is defined by the interval between two successive expressions of oestrus or two successive ovulations. While the average duration of the goat oestrous cycle is of 21 days, its length is highly variable. A study with Alpine goats during the breeding season recorded 77% cycles of normal duration (17–25 days), 14% were short cycles (8 days in average) and 9% were long cycles (39 days in average). The relative high frequency of short cycles is characteristic of goats and increases when ovulation is induced either just before or during breeding season. This proportion can be modulated by environmental factors such as photoperiod and nutrition. During the oestrous cycle, ovaries undergo a number of morphological (follicular recruitment and growth), biochemical (follicle maturation) and physiological (endocrine regulations) changes leading to the ovulation. These cyclical changes in the gonads are referred to as the ovarian cycle. Follicular growth evolves in a wave-like manner throughout the cycle. A follicular wave is characterized by the sequence of three gonadotropin-dependent events in follicular growth: recruitment, selection and dominance. Studies using repeated ultrasonography suggest that there are between two and six waves of follicle development during oestrous cycles in goats with three or four waves being the most prevalent. The last wave provides the ovulatory follicle. When double ovulations occur they are usually of follicles derived from the same wave, but in a few cases they derive from two consecutive follicle waves. The oestrous cycle is classically divided in two phases: the follicular phase and the luteal phase. The follicular phase corresponds to the wave of follicle development providing the ovulatory follicle and involves maturation of gonadotropin-dependant follicles until ovulation (terminal growth). During the follicular phase, FSH secreted by the pituitary gland stimulates follicular growth. A cohort of gonadotropin-dependant antral follicles of 2–3mm of diameter is recruited and follicles enter their terminal growth. Only 2–3 of these follicles reach 4mm diameter and are selected to enter the dominance phase. Under the influence of LH, they reach the pre-ovulatory stage (6–9 mm), while subordinate follicles degenerate (follicular atresia). The increase in peripheral concentrations of oestradiol 17\_\alpha, secreted by bigger follicles, induces oestrous behaviour and acts as a positive retrocontrol on the gonadotropic axis. The consequent increase in GnRH secretion induces the pre-ovulatory LH surge which induces ovulation 20–26 h later and subsequently luteinization of follicular cells. The beginning of the follicular phase, before overt oestrous behaviour is observed, is also referred to as the prooestrus. The oestrous phase includes events from overt oestrous behaviour to ovulation. Both season and nutrition are known to affect
the ovulation rate, especially in the Angora breed. Angora goats typically have a single ovulation under most production conditions but may have two under very good nutritional conditions. Average ovulation rate is reported as 1.7 in Boer goat, 1.5 in local Maure goat and probably much higher in Chinese Matou goat, which have an average litter size of 2.1. The luteal phase starts from the time of ovulation. About 5 days after the onset of oestrus, cells of the ovulating follicle turn into luteal cells and form the corpus luteum (CL). They secrete progesterone causing its concentrations to increase and remain at a high level (>1 ng/ml) during 16 days. During this luteal phase, gonadotropin-dependant follicular growth continues in a wave-like manner but progesterone inhibits ovulation. At the end of the luteal phase, 16–18 days after oestrus, prostaglandin F2α-secreted by the non-gravid uterus induces the CL regression – called luteolysis – and the decrease of progesterone secretion. The decrease of plasma concentrations of progesterone gradually removes the inhibition of gonadotropic hormones secretion and a new follicular phase then commences. The luteal phase is also called the postoestrous period, which can be divided in metoestrous, when peripheral concentrations of progesterone begin to rise, and dioestrous, when peripheral concentrations of progesterone are high up to the start of luteolysis.

**Oestrous behaviour**

Oestrous behaviour includes two phases: proceptivity and receptivity. Proceptivity consists in seeking out and stimulating the male partner. Receptivity consists in the expression of the immobilization reflex in response to male nudges, inducing serial mounting and copulation. At the beginning of oestrus, proceptivity always precede receptivity, then both behaviour components are expressed simultaneously. The duration of oestrus behaviour is about 48 h but varies from 24 h to 48 h depending on age, individuals and breeds, season and the presence of a male. Angora goats and Mossi goats are known to have a short oestrus lasting only 22 h and 20 h, respectively. Creole goats exhibit 27 h of oestrous behavior and French Alpine goats are reported to experience a 31-h oestrus. In Boer goats, the mean duration of oestrous period is about 37 h and it is of about 58 h in Matou goats in Central China. The interval from the beginning of oestrus to the LH surge varies with breeds and individuals: 14.5 h in Alpine, 14–22 h in the Mossi goat in Burkina Faso and as short as 8 h in Boer goat. The exact timing of ovulation relative to the onset of oestrus is variable, ranging from 36 h to 37 h, and is reported generally as occurring towards the end of standing oestrus. Continuous presence of a male and service during the oestrus period may reduce the duration of oestrus although it did not affect ovulation times or ovulation rates in Nubian dairy goats.

**Breeding and gestation**

Copulation occurs during oestrus, therefore usually before ovulation. Hence, sperm progressing through the female genital tract may be present in the oviduct by the time of ovulation. Meanwhile, other sperm is retained in the cervix where preservation conditions are good (up to 3 days) and released continuously in the uterus where survival is limited to about 30 h. The primary mode of sperm transport is by contractility of the female reproductive tract, though sperm motility might be important in the cervix for migration through the cervical mucus. Ova may remain viable for 10–25 h. Fertilization occurs in the ampullae of the oviduct a few hours after ovulation. The fertilized ovum migrates down the oviduct while undergoing successive divisions. The embryo reaches the uterus 4–5 days after oestrus at an early morula stage. Migration of the ovum is the result of combined movements of ciliated epithelial cells in the oviduct, peristaltic activity of muscular layers and a liquid
current from the infundibulum to the uterus. Implantation of the embryo is observed 18–22 days after the onset of oestrus. While in sheep, the placenta becomes the primary source of progesterone after 2 months of gestation, in goats, the presence of a functional corpus luteum is indispensable throughout gestation. The placental production of progesterone in goats is unable to maintain pregnancy after ovariec-tomy or luteectomy. Parturition in goats is preceded by a drastic decline in progesterone 12–24 h before the beginning of labour. Gestation length averages about 149 days but it may vary a little between breeds. Breed of dam, litter weight, breeding season and parity effects on gestation length have been observed by many authors. The number of kids born and gender of kids were not a significant source of variation affecting this trait. Granadina goats had the shortest gestation (149.0±0.31 days), whereas Toggenburg (151.7±0.28 days) and Alpine (151.4±0.46 days) had the longest. Boer goats also have a shorter gestation: 148.2±3.7 days. Gestation of goats bred in summer was 1 day longer than those mated in autumn and there was a progressive reduction of gestation length as parity increased. Hydrometra or pseudopregnancy is a well-known ailment in goats, though its causes remain unclear. It is described as the accumulation of aseptic fluid within the uterus associated to high peripheral concentrations of progesterone (similar to that of pregnant goats) due to one or more persistent corpora lutea. The incidence of hydrometra has been described in breeds of dairy goats in The Netherlands and in France and varies from 3% to 20%. The frequency is significantly higher in older goats than in yearling goats. Pseudopregnancy seems to occur more often in animals bred out of the natural breeding season and/or after an induced ovulation. Systematic diagnosis of hydrometra by ultrasonography before hormonal treatment and artificial insemination improve fertility results.

**Seasonal sexual activity**

Reproduction in goats is commonly described as seasonal with differences in seasonality between breeds and locations. The onset and length of the breeding season in goats is dependent on a number of factors: latitude and climate, breed, physiological stage, presence of a male, breeding system but mainly photoperiod. The main environmental factor affecting seasonal breeding in small ruminants is the annual change in day length. Photoperiodic control of reproductive patterns is mediated through circadian rhythmic secretions of melatonin by the pineal gland during darkness which influences the gonadotropin-releasing hormone pulse generation and the hypothalamic–pituitary–gonadal feedback loop. Animals bred in tropical and equatorial regions subjected to less change in photoperiod and temperature, display a longer breeding season than those bred in temperate and Polar Regions which exhibit more distinct seasonal effects.

**At high latitudes**

The sexual activity in female goats can be assessed by their spontaneous ovulatory activity and demonstration of sexual behaviour. Two distinct periods are observed throughout the year in temperate latitudes: a period of deep anoestrus, when neither oestrous behaviour nor ovulations are noted, and the breeding period, with both oestrous behaviour and cyclic ovarian activity observed. During the transition periods, anoovulatory oestrus or silent ovulations (ovulation not accompanied by oestrous behaviour) can also be observed. Seasonal breeding is observed in most breeds of goats originating from high latitudes (>35°) and in some local breeds from subtropical latitudes (25–35°). In temperate regions the breeding period is observed in the fall and winter. In France (45° North Latitude), the breeding season starts in September, when day length is declining, and persists until March. In Australia (10–39° South
Latitude), goats have a brief period of spontaneous ovulatory activity, from April to August, centered on the winter solstice with a peak in June. In the Alpine and local goats bred in subtropical Mexico, the breeding season begins in the early autumn and ends in the late winter. With increasing latitude, for example in Swedish Landrace goat, the breeding season tends to be restricted to the autumn months and most kids are born in spring. Exposure to males can extend the period of ovulatory activity both before and after the period of spontaneous ovulations. In Australian goats, exposure to bucks, either continuous or intermittent, has been shown to extend the ovulatory period a month before and after the normal timescale (March–September).

At lower latitudes

In equatorial, tropical and subtropical regions, changes in day length are less pronounced. Seasonality in reproduction is therefore less marked and most local goats in the tropics have the ability to breed all year-round and have a relatively short post-partum anoestrous. However, environmental factors (forage availability and temperature changes) have a strong influence which often do not allow these potentials to be fully expressed. In particular, insufficient nutrition is often responsible for the appearance of prolonged anoestrous and anoestrous periods, a reduction in fertility and prolificacy and also causes an elevated spring mortality rate. The most suitable times for mating and kidding are determined by climatic or management factors. For example, in some regions, ovulation is thought to be induced by monsoon rains so as to delay kidding until after the monsoon is over. Year-round kidding potential has been observed in tropical goats like Creole goats bred in Guadeloupe, though not homogeneously distributed throughout the year: a peak period still occurs in spring with few kidding recorded in autumn. There is inevitable confounding between the effect of breed and latitude. Goat breeds are used rather regionally and one breed is rarely present in multiple climatic regions. Indeed, animals originating from temperate regions that have undergone a long period of adaptation to tropical photoperiod, experience a longer breeding season than they did in their temperate region. Similarly, animals from tropical areas such as Creole goats, which exhibit year-round reproduction, can become restricted in their breeding season when treated with an artificial temperate-type photoperiod.

Control of sexual activity and optimization of fertility results

Techniques used to control reproduction in goats allow greater distribution of milk and meat production throughout the year, thereby meeting the supply needs of industries and consumers. Controlling goat reproduction offers three main advantages: (1) the choice of a kidding period at a precise season of the year (determined by forage availability or by marketing of the products), (2) the synchronization of kiddings over a limited period of time (labor optimization, reduction in kid mortality, constitution of homogeneous feed lots of mothers and kids) and (3) the management of genetic resources (genetic selection, storage of genetic material).

Hormonal treatment

Hormonal regimens based on progestagens, eCG (equine chorionic gonadotropin) and/or prostaglandins have been established for over four decades allowing oestrous and ovulation synchronization during both the breeding and non-breeding seasons. In France, the treatment consists of the deposition of a vaginal sponge impregnated with a progestagen (20–45mg fluorogestone acetate) for 11 days. An intramuscular injection of a PGF2 analog (50 g cloprostenol) and 250–600 IU of eCG (dosage is dependent on parity, season and milk production level) is made 48 h before sponge removal. Artificial insemination is carried out 43–45 h after sponge removal. For out-
of-season AI with frozen-thawed semen, this treatment allows a conception rate of about 60–65%. It is currently used on 95% of inseminated dairy goats in France, but is also used to facilitate natural mating (in combination with photoperiodic treatment when used out of the breeding season). This treatment can be used at any time of the year, independently of the strength of seasonality. Other progestagens and other progestagen dispensers have been used in different countries including vaginal sponge impregnated with 60mg medroxyprogesterone acetate, subcutaneous implants containing 3–6mg Norgestomet and controlled internal drug releasing device (CIDR) containing 330mg progesterone.

**Photoperiodic treatments**

Seasonality of reproduction in goats is strongly dependant on photoperiod. In temperate and subtropical regions, out-of-breeding season breeding can be achieved using strategies based on manipulation of the photoperiod. Following extended exposure to decreasing day length, animals become photo-refractory to the short day stimulus and will cease cyclic activity, unless a period of long day photostimulation is supplied. Photoperiodic treatments are based on alternation of long and short days. First, the animals are subjected to long days (provided by artificial lighting in winter or by natural days in spring and summer) in order to prepare them to respond to the stimulatory effects of subsequently administered short days. Under field conditions, short day effects are easily provided by melatonin implants. These photoperiodic treatments can induce sexual activity in males and females similarly to hormonal treatment in females. Photoperiodic treatments can induce ovulation over several weeks but cannot synchronize ovulation sufficiently to facilitate AI. Photoperiodic treatments are generally combined with hormonal treatments or the buck effect for synchronizing ovulations.

**Buck effect**

Does and bucks are sensitive to their social environment, which can be used to manage their reproductive cycle. The so-called male effect is a technique to stimulate the sexual activity in seasonally anovulatory goats. Most female goats have a short ovarian cycle of 5–7 day-length following the introduction of bucks, followed by a second ovulation associated with oestrous behaviour and a normal luteal phase. One of the major factors affecting the efficacy of response to the male effect depends on the strength of seasonality of the female and male goats. In this respect, the response to the male effect varies within breeds through the seasonal anoestrous period, and among breeds from different latitude origins. For example, in breeds exhibiting moderate seasonality, such as the Creole goats of Guadeloupe Island, introduction of the male may induce highly fertile ovarian activity in anovulatory goats throughout the year. In contrast, when used alone in highly seasonal breeds, the male effect can only advance the onset of the breeding season by a few weeks; it does not satisfactorily induce full sexual activity in the middle of the anoestrous period. Depending on breed and/or on anoestrous period, the pre-treatment of females and/or males with photoperiod may be necessary to optimize the response to the male effect. For instance, in Alpine and Saanen breeds in France, the treatment of males and females with artificial photoperiod is necessary to improve the response to the male effect. Under these conditions, most does exposed to male’s ovulated (99%) and 81% kidded. In subtropical Mexico, the main critical factor for success of the male effect is the use of sexually active males. Bucks, but not females, are thus treated with long days followed by natural days or melatonin to improve the efficiency of the male effect and can stimulate ovulation and oestrous behaviour in female goats better than
untreated males. Availability of sexually active males allows producers to manipulate the annual breeding season according to consumer demands, without the restraint of also keeping females in confinement for teasing.

**Adapted nutrition**

Most characteristics of the reproductive cycle can be modulated through adapted nutrition. Nutritional strategies have recently been developed based on knowledge of precise nutritional needs for each stage of the reproductive process and interaction between metabolic status and reproductive performance. Both in sheep and goats, a long-term increase in body weight as well as a timed supplementation are known to affect folliculogenesis. Targeted nutrition can thus increase potential litter size by optimizing ovulation rate. The total number of offspring produced per doe can also be increased with planned nutrition to advance puberty. This was observed in Savannah Brown goats and seemed independent of their body weight. In addition, it has been shown that the response of ewes and goats exposed to males can be influenced by their nutritional status. The proportion of goats that displayed estrous behaviour, their ovulation rate and pregnancy rate in response to male effect were all greater in supplemented females compared with the non-supplemented group. During pregnancy, nutrition can also affect both embryo survival and foetal programming of adult performance. Nevertheless, these tools can only increase reproductive performance within biological limits and should be adjusted to the considered breed and environment.

**BUFFALO**

The domestic water buffalo (Bubalus bubalis) is an important livestock resource in many countries of Asia, the Mediterranean region and Latin America. Water buffalo are classified into two main types: the river type located in South Asia and the swamp type spread across the South-East Asian region. The Mediterranean buffalo, which some consider to be a third type, is derived from the river type. The main breeds of dairy buffalo belong to the river type and include the Murrah, Surti, Jafarabadi and Nili-Ravi. The swamp type has no specialized breeds but selective breeding in some countries has resulted in populations with characteristic features.

**Puberty**

Buffalo heifers usually attain puberty when they reach about 55–60% of their adult body weight, but the age at which they attain puberty can be highly variable, ranging from 18 to 46 months. The factors that influence this are genotype, nutrition, management, social environment, climate, year or season of birth and diseases. A review of studies from many countries shows that under favourable conditions river type buffaloes exhibit first oestrus at 15–18 months of age, while the swamp type do so at 21–24 months. The body weight at which puberty is attained is strongly influenced by genotype and is around 200–300 kg for the swamp type and 250–400 kg for the river type. Although buffalo attain puberty later than cattle, they have a longer reproductive life, which tends to compensate for this early economic disadvantage.

**Ovaries, follicles and corpora lutea**

The ovaries of post-pubertal buffalo heifers have a reservoir of only 10,000–20,000 primordial follicles compared with over 100,000 in cattle. The mature ovaries are smaller than in cattle, weighing around 2.5 g when inactive and 4 g when active, with fewer tertiary follicles. When palpated per rectum, mature follicles in swamp buffalo rarely exceed 8 mm in diameter, tend to protrude from the surface of the ovary and can be mistaken as an early developing corpus luteum. The corpus luteum is smaller than that in cattle, often does not protrude markedly from the surface of the ovary and
sometimes lacks a clear crown. These characteristics make accurate identification of ovarian structures by rectal palpation in buffalo more difficult than in cattle. Ultrasonic imaging indicates mature follicles range in size from 1.3 to 1.6cm in diameter and mature corpora lutea from 1.2 to 1.7cm in diameter.

**Reproductive anatomy**

The reproductive tract of the water buffalo (Bubalus bubalis) is quite similar to that of domestic cattle (Bos taurus and Bos indicus). The tubular genitalia of the buffalo are generally more muscular and firmer, and the uterine horns are more coiled than those of the cow. The body of the uterus is much shorter (1–2 cm) than that of the cow (2–4 cm). The cervix of the water buffalo is smaller than that of the cow (length 3–10 cm, diameter 1.5–6.0 cm) and its canal is more tortuous, which probably accounts for less dilation of the external os during estrus. The average number of cervical folds in water buffalo is three. The inactive ovaries of the mature water buffalo are smaller (3.0 cm _ 1.4 cm _ 1.0 cm; 2.9–6.1 g) versus (3.7 cm _ 2.5 cm _ 1.5 cm; 5–13 g) in the cow. There are differences due to variations in breed, environmental conditions, season, and management practices.

**Reproductive patterns**

Buffalo are polyoestrous and are capable of breeding throughout the year. However, in many countries a seasonal pattern of breeding activity, and consequently calving, has been observed. In tropical locations where photoperiod is relatively constant, annual changes in rainfall appear to influence oestrous cyclicity, with availability and quality of herbage related to this cyclical reproductive pattern. In the dry zone of Sri Lanka, buffalo kept under free grazing commenced oestrous activity some 2–3 months after the onset of monsoonal rains, followed by conceptions that result in a peak calving season 10 months later. Similar effects of climate and nutrition on reproductive patterns have been observed in India and the Amazon region of Brazil. Heat stress during the hot summer months in India is a major cause of anoestrus in buffalo and is associated with elevated blood concentrations of prolactin, which is thought to influence ovarian activity as well as cause sub-fertility and repeat breeding by decreasing progesterone secretion. In temperate regions such as Italy, however, where buffalo are fed with a constant balanced diet, a distinct seasonal reproductive pattern is also found, and the inference from a series of studies is that seasonality is influenced by photoperiod and mediated by melatonin secretion.

**Estrous cycle**

The duration of the oestrous cycle in buffalo is similar to that in cattle, ranging from 17 to 26 days with a mean of around 21 days (Jainudeen and Hafez, 1993). However, there is greater variability of oestrous cycle length in buffalo, with a greater incidence of both abnormally short and long oestrous cycles, attributed to various factors including adverse environmental conditions, nutrition and irregularities in secretion of ovarian steroid hormones. The duration of oestrous is similar in river and swamp buffalo, varying between 5 and 27 h, and ovulation occurs about 24–48 h (mean 34 h) after onset of oestrus, or 6–21 h (mean 14 h) after the end of oestrus. In hot climates duration of oestrous tends to be shorter and signs of oestrus may be exhibited only during the night or early morning. In Italian buffalo different durations of oestrus have been observed and categorized as short (<12 h), medium (13–24 h), long (24–48 h) and very long (>48 h). In the short and medium oestrous cycles ovulation occurred after the end of oestrus, around 6–72 h and 24–60 h after the onset of oestrus, respectively. In some of the long and very long oestrous cycles ovulation occurred before the end of oestrus. Compared to cattle, estrous behavior in water buffalo is
much more subtle, and homosexual behavior, i.e. females mounting females, is rare. Secondary signs such as swollen vulva, reddening of the vulvar mucosa, mucous vaginal discharge, and frequent urination are not reliable indicators of estrus. Ovulation occurs 30 h after the onset of estrus (range 18–45 h). Twin ovulations are rare. The diameter of an ovulatory follicle is _10 mm. The diameter of the mature CL ranges from 10 to 15 mm versus 12.5 to 25.0 mm in the bovine. The ovulation papilla, or crown, of the CL does not protrude much beyond the surface of the ovary, making it more difficult to identify by palpation per rectum. The CL of pregnancy is invariably located ipsilateral to the gravid horn. The growth, selection, regression and ovulation of follicles were monitored by ultrasound in 30 river buffaloes throughout a spontaneous estrous cycle during the breeding season (autumn in Brazil). As in cattle, follicular growth occurred in waves in buffaloes. Two-wave cycles were most common (63.3%) followed by three wave cycles (33.3%) and a single wave cycle (3.3%). The number of waves influenced the length of the luteal phase and the estrous cycle. The changes in concentrations of progesterone in blood and milk during the oestrous cycle are similar to those in cattle, but the peak concentration is relatively less. Progesterone concentration in fat-free milk is usually below 1 nmol/l (∼0.3 ng/ml) during the follicular phase of the oestrous cycle and ranges from 3 to 12 nmol/l (∼1–4 ng/ml) during the luteal phase and pregnancy. For assessing cyclic ovarian activity and for early diagnosis of non-pregnancy, progesterone concentrations above 3 nmol/l are considered to be indicative of the presence of luteal function and those below 1 nmol/l are considered indicative of the absence of luteal function, with intermediate values being considered inconclusive. The concentration of oestradiol-17 in blood during the follicular phase of the oestrous cycle also appears to be relatively less than that in cattle. Although this has been suggested as a possible reason for the lesser intensity of oestrus exhibited by buffalos, studies on Italian buffalo have shown no differences in the endocrine profiles of those with overt and oestrus without an associated ovulation.

**Estrous behavior**

A major difference between buffalo and cattle is that behavioral signs of estrus are less overt than in the former, with homosexual behavior between females being rare. In the absence of a bull, the main behavioral signs are restlessness, bellowing and frequent voiding of small quantities of urine, but these are not consistently exhibited by all animals. When a bull is present, however, the bull will show increasing interest in a cow that is approaching estrus, and the cow will stand to be mounted by the bull during estrus. During periods of greater ambient temperature the duration of oestrus may be shorter and the estrual signs exhibited only during the night or early morning.

**Estrus detection**

Covert or silent estrus is the single largest factor responsible for poor reproductive efficiency in buffalo. Estrus detection is a prerequisite for efficient reproductive management. Accurate estrus detection is essential when hand-mating to selected sires is practiced. To compensate for the lack of overt estrous behavior among females, estrus can be detected with the aid of teaser animals, or pedometers, or it can be induced with hormonal treatments. Teaser animals can be bulls with a lateral deviation of the penis and an epididymectomy, or androgenized females. Vasectomized bulls per se are less desirable due to the risk of spreading venereal diseases. Teaser animals should be fitted with a chin-ball marking device to identify the animals in estrus.
THE BITCH:
Reproductive activity in the bitch differs from the polycyclic pattern of other species in that there are no frequent, recurring periods of heat. All bitches have a prolonged period of anoestrus or sexual quiescence between successive heats irrespective of whether they have been pregnant or not; this pattern has been described as monocyclic. The average interval between successive oestrous periods is 7 months, but it is variable, and there is some evidence that the breed of the bitch can have an effect. The oestrous cycle is traditionally divided into four phases:

Pro-oestrus. The bitch has a true pro-oestrus characterised by the presence of vulval oedema, swelling and a sanguinous discharge. Some fastidious bitches show no evidence of discharge as they are continually cleaning the perineum. The bitch is attractive to males but will not accept the male.

Oestrus. The bitch will accept the male and adopts the breeding stance. The vulva becomes less oedematous and the vulval discharge becomes clearer, less sanguinous and less copious. The duration of pro-oestrus and oestrus combined is about 16 days, i.e. 9 days each. However, this can be very variable, some bitches showing very little sign of pro-oestrus before they will accept and stand for the dog and others producing a copious sanguinous discharge during true oestrus. Some bitches also show evidence of sire preference, which can affect the timing. Ovulation usually occurs 1 or 2 days after the onset of oestrus, although, using laparoscopy, it has been observed that some follicles continue to ovulate up to 14 days later.

Metoestrus. This stage starts when the bitch ceases to accept the dog; however, there is dispute about its duration. Some consider that it ends when the corpora lutea have regressed at 70–80 days whilst others measure it in relation to the time taken for repair of the endometrium, 130–140 days.

Anoestrus. At the end of metoestrus the bitch passes into a period of anoestrus without any external signs. The same is also true after parturition following a normal pregnancy. This phase lasts about 3 months before the bitch returns to pro-oestrus.

Bitches are monoestrous, typically non-seasonal, polytocous, spontaneous ovulators and have a spontaneous luteal phase similar in length to or a bit longer than the 64±1 day luteal phase of the 65±1 days of pregnancy followed by an obligate anoestrus before the next 2 to 3 week “heat” period (Table 1). Inter-oestrus intervals of 5–12 months, typically 6–9 months, range from highly variable to regular within bitches, and averages do not vary significantly or consistently between pregnant and non-pregnant cycles. Puberal estrus occurs variably at 6–14 months in most breeds, with means positively correlated with breed size. The canine cycle is classically divided into 4 phases—a 5–20 day prooestrus, 5–15 day estrus, 50–80 day metoestrus (post-estrus portion of luteal phase), and anoestrus typically lasting 80–240 days. These phases reflect, respectively, follicular phase rise in estrogen, the initial luteal phase rise in progesterone and decline in estrogen, the remainder of the luteal phase, and the interval between loss of luteal function and onset of next cycle. Timing within the 160–370 day cycle has been variably reported in days post prooestrus onset, estrus onset, metoestrus onset, preovulatory LH peak or LH surge. The latter is used as day 0 in this review unless otherwise noted. Levels of hormones are primarily those observed in the author’s laboratory using previously reported assays and canine gonadotropin standards.

Endocrine mechanisms of the canine cycle are not unlike those of other mammals as interpreted from hormone profiles and results of experimental manipulations. Steroid assays established for ruminants and humans have been adapted to canine serum and plasma using sample extraction or direct assays with samples from ovariectomized
animals controlling for non-specific interference. LH, FSH and prolactin are assayed using either heterologous assays based on antisera to ruminant or rodent hormones or homologous assays, with purified canine hormone preparations as standards. Ovulation occurs in response to an abrupt end-of-proestrus gonadotropin surge resulting in a 1–3 day elevation in LH and a 1–4 day elevation in FSH. Ovulation has been timed to occur about 48–60 h after the LH surge. In bitches, and in contrast to most other mammals, oocyte maturation occurs in the distal uterine tubes ~2 days post ovulation, as in foxes. In humans and many rodents, the preovulatory increase in follicular progesterone production is accompanied by a rise in peripheral progesterone useful for timing ovulation and this is similar in the bitch. Likewise bitches as for many rodents require prolactin in addition to LH for luteotrophin. There is no acute luteolytic mechanism and hysterectomy has no effect on CL function. The lengthy luteal phase results in detectable mammary enlargement that in a proportion of bitches can result in a clinical condition, overt pseudo-pregnancy, characterized by gross mammary enlargement accompanied by lactogenesis and lactopoiesis. Pregnant cycles are characterized by enhanced progesterone secretion effected by pregnancy-related increases in prolactin after implantation, perhaps in response to increased relaxin; parturition occurs in response to an abrupt luteolytic rise in systematic PGF immediately prepartum.

Proestrus
Proestrus occurs when external signs of increased estrousization are first observed as vulval swelling (edema) usually accompanied by serosanguinous vulval discharge. Proestrus averages 9 days and is characterized by progressive increases in: vulval size and turgor; vaginal epithelial proliferation, cornification, and edema; epithelial cell numbers in vaginal smears; and, vaginal secretion of male-attracting pheromones. Vaginal smear epithelial cell profiles change from being dominated by parabasal cells (accompanied by varying numbers of neutrophils), to being dominated successively by small intermediate squamous cells, large intermediates, and then large cornified cells until finally comprised of entirely (98–100%) cornified cells and virtual absence of neutrophils that no longer traverse the thickened epithelium. Cornification peaks variably at 1–6 days before the LH surge. The serosanguinous discharge involves serous fluid containing intact and lysed erythrocytes and their hemoglobin originating by diapedesis in the uterus; there are no reliably characteristic changes in their number or appearance. Morphoscopically, the mucosa appears edematous, changing progressively from pinkish to white, with serosanguinous fluid on the surface and in deepening vagina folds that become more prominent in both axes, yielding a smooth cobble-stone appearance. There is a progressive decline in aggressive response to interested males. Refusals of mounting attempts progressively change from aggressive to ambivalent to playful to passively lying down. Male attraction involves pheromone secretion, and methyl p-hydroxybenzoate was identified as a sex attractant in vaginal secretions of estrous bitches. Small amounts applied to the vulva cause males to become aroused; studies of low ug amounts were discontinued because of hyper-responsiveness and colony disruption (Concannon, unpublished). Serum estradiol increases throughout, from 5 to 15 pg/ml (~20–55 pmol/l) initially, to reach peaks of 40–120 (mean 70) pg/ml (~150–450, mean 255 pmol/l). Proestrus ends with the onset of receptive behavior typically occurring 0.5–3 days after the peak in estradiol and within a day of the preovulatory LH surge. Endocrinologically, physiological proestrus ends with the preovulatory LH surge.

Estrus
Estrus behavior is characterized by proactive receptivity to mounting by males and increased male-seeking behavior. Estrus lasts a variable length of time, wanes slowly or rapidly after 5–10 days (mean 9 days) but can persist to some extent well beyond the day 8 end of “fertile estrus”. Clinically defined, estrus lasts until vaginal anatomy and cytology no longer reflect full or maximal cornification but rather extensive regional desquamation, appearance of underlying non-cornified cells, and epithelial thinning with migration of neutrophils into the lumen, changes that typically occur 6–11 days (average 8 days) after the LH surge. Physiological estrus onset has no distinct cytological correlates, but anatomically is reflected in initial wrinkling and crenulation of the endoscopically viewed vaginal mucosa±1 day from the LH surge, as a response to the sharp decline in estrogen: progesterone ratio. Maximal crenulation occurs by day 4–5. In some instances, fertile cycles with normal endocrine profiles can occur with estrus behavior onset as early as 2 days before the LH peak, as late as 6 days after the LH peak, or not at all. Estrus failure has been documented in otherwise fertile cycles by the use of properly timed AI and resulting pregnancies. Some of the variation in onset or occurrence of behavioral estrus within and among bitches involves variation in responses to different males, in behavior being scored in the absence vs. presence of males, and in definitions of estrous onset varying among “receptive reflexes”, “standing”, “intromissions” and “first ejaculatory copulation or copulatory lock”, which in some instances all occur within the day and in others occur completely or incompletely in sequence over several days. Estradiol continues its decline from peak values of late proestrus to intermediate values of 10–20 pg/ml (~40–90 pmol/l). Serum progesterone rapidly increases above 1–3 ng/ml (3–6 nmol/l) during the preovulatory LH surge, and immediately (or after a 1–3 days pause) rapidly increases further, reaching 10–25 ng/ml (~30–80 nmol/l) by day 10, at or shortly after the end of estrus. Estrus in the bitch occurs in response to the decline in estradiol that normally begins shortly before the LH surge and continues throughout estrus. Estrus onset is facilitated synergistically by the rapid rise in progesterone resulting from the LH surge. Objectively scored estrus behavior induced by estrogen withdrawal in estrogen-treated ovarietomized bitches has been reported to be more intense, rapid, and synchronous when progesterone was administered at the time of estrogen withdrawal.

Met estrus (metestrus)

Metestrus, the post-estrus portion of the luteal phase, was initially defined behaviorally as starting when estrous behavior ceases. Using morphological criteria, metestrus begins when a day 6–11 “metestrus” vaginal smear or “metestrus” vaginal mucosa is first detected. Metestrus is considered to last until evidence of the ongoing luteal phase becomes minimal. The end of metestrus, and anestrus onset, are variably defined as when uterine endometrium has undergone histological “repair”, when mammary enlargement in response to luteal phase progesterone recedes, and most often in recent decades, when serum progesterone declines to levels persistently below 1 or 2 mg/ml (~3–6 nmol/l). Serum progesterone increases to peaks of 15–80 ng/ml (~50–250 nmol/l) between cycle day 20 and 35, and slowly declines thereafter, going below 1 ng/ml (~3 nmol/l) by day 55–90 (mean 70). Estradiol is variable at intermediate values of 15–30 pg/ml (~15–110 pmol/l) with profiles to some extent paralleling those of progesterone, higher in mid-luteal phase and then declining. The term “diestrus” is used in some veterinary texts as a substitute for and synonymous with metestrus in bitches, thereby applying ‘diestrus’ across species to generally refer to the period of luteal function and avoiding possible misconceptions that ‘metestrus’ might refer to only a short period after estrus as in descriptions of artiodactyls and
rodent cycles. With ‘diestrus’ used for bitches in that context, unlike in other species, estrus and not diestrus (metestrus) still occupies most of the growth phase of the canine CL, and an intervening anestrous and not diestrus immediately precedes proestrus.

Anestrous
Canine anestrus involves the absence of overt evidence of ovarian activity, is considered to be “obligate” lasting a minimum of 7 weeks after progesterone declines below 1–2 ng/ml, and averages 18–20 weeks. It may initially be important for the normal endometrial repair that is completed around day 120–130. The apoptotic index and percent of degenerated epithelial cells in the endometrium are high during the mid-luteal phase, low in early anestrus and absent by day 120. Vaginal cytology shows sparse numbers of parabasal cells (and degenerate “squames”) and variable but modest numbers of neutrophils. The vaginal mucosa appears thin and red with visible capillaries; the surface is easily traumatized and vaginal cytology difficult to monitor without inducing bleeding with spurious erythrocytes in smears. Serum estradiol is reported to be variable but generally low at 5–10 pg/ml (∼15–30 pmol/l). Basal LH is low (<1–2 ng/ml) between sporadic, variable height and often large pulses (3–30 ng/ml) at intervals of 7–18 h or longer. FSH is high (60–400 ng/ml, mean 140) between sporadic pulses slightly above elevated baseline that when detectable are typically concomitant with LH pulses. Serum progesterone remains below 1 ng/ml (under ∼4 nmol/l), with a nadir near 400 pg/ml (∼1500 pmol/l) at 30–40 days before proestrus.

Ovulation
Determining the time of ovulation is often critical in breeding management, timing AI, monitoring ovulation-induction, and reproductive experimentation. Where access to rapid LH assays is not available, ovulation is best timed as occurring 2 days after the first abrupt rise in progesterone of > 0.5 ng/ml and reaching ≥0.9 ng/ml, an event that occurs concomitantly with the LH surge in over 95% of cycles. When early and frequent measurements are not available, the first day with concentrations ≥5 ng/ml is often considered indicative of ovulation in breeding management. A rapid reduction in vulval turgor due to preovulatory declines in the E:P ratio typically indicates ovulation is either imminent or has just occurred. Similarly, intense crenulation of the vaginal mucosa due to declining estradiol is informative, as it becomes maximal 2–3 days after ovulation, and recedes thereafter. Ovarian ultrasound can also determine the time of ovulation with considerable accuracy, based on the transient 1–2 day marked increase in echogenicity of previously anechoic follicles at ovulation, followed by a return of anechoic structures, i.e., day 4 “antral” CL. Whether echogenicity at ovulation is due to bleeding, follicle collapse, or change in follicular fluid composition is not known. The LH surge to ovulation interval is characterized by a rapid increase in follicle mural cell luteinization, in growth of theca and blood vessels, abrupt increases in serum progesterone and 17-hydroxyprogesterone, and typically further declines in estradiol. Increased follicular progesterone is likely to be critically involved in ovulation, as in other species. Progesterone’s participation in positive feedback cascades involving PGE and/or oxytocin production and increases in metalloprotease, as in the cow, have not been studied in bitches. Details of oocyte maturation (MII at 48–54 h post ovulation), fertilization and early embryo development have been reported and elegantly reviewed. Interestingly, peripheral cumulus cells undergo mucification before ovulation but mucification of the compact inner-most cumulus layers and their expansion from the zona are delayed
24–36 h or more after ovulation and appear indicative of the cytoplasmic maturation of the oocyte.

Reproductive tract, mammary development, and pseudopregnancy

The ovary, fully enclosed in a bursal membrane with a small bursal slit, is typically visible trans-membrane in young animals, but often not in older animals due to bursal fat. The long uterine horns become serpentine during rapid growth in estrus and early luteal phase. The short fundus provides for translocation of ova in estrus. The single cervix is oriented with the os facing nearly ventrad into the anterior vagina, cranial to an often prominent dorsal-median fold (dmf) but visible vaginiscopically, and amenable to transcervical insemination with varying difficulty. The caudal aspect of the dmf may present as a “false cervix”, with the occluded vaginal lumen appearing like the cervical os of some species. Oviduct and endometrial responses to estrogen and progesterone appear similar to those described in rodents, and maximal endometrial development requires sequential exposure to estrogen and progesterone, as occurs in normal cycles. Sex steroid receptor changes in reproductive tissues show cyclic changes similar to other species; recent detailed analyses of canine endometrial cells have been extensive. Endometrial ER and PR increase in proestrus, decrease in estrus and metestrus, and are replenished in anestrus. Mitotic indices of endometrial stroma, blood vessels and surface epithelium are maximal under estrogen influence in proestrus and decline thereafter, whereas indices for glandular epithelium peak in estrus and metestrus under the early influences of progesterone. Mammary and uterine disease is more common in dogs than in other domestic species, perhaps due to progesterone sensitivity, absence of routine pregnancy, and use of progestins for contraception. Luteal progesterone, an exogenous progesterone alone, can cause extensive endometrial hyperplasia, and often cyclic endometrial hyperplasia (CEH) that can support opportunistic infection and pyometra. Researchers in Osaka found that progesterone suppresses peripheral cellular immunity in metestrus. CEH progressing to pyometra is the most common medical problem of domestic bitches and is usually managed surgically (ovariohysterectomy). Medical management in breeding bitches involves uterotonic and luteolytic effects of PGF injections, using PGF alone, or combined with prolactin-lowering dopamine agonist doses synergistic in inducing luteolysis or with antiprogestin treatment that enhances progesterone withdrawal. The latter causes pre-partum-like changes including opening of the cervix, and thereby facilitates evacuation of uterine contents in response to PGF. In every normal cycle, sequential exposure to estrogen and progesterone causes significant mammary growth and enlargement detectable by palpation but is otherwise typically not obvious. A clinical condition, “overt” or “clinical” pseudo-pregnancy, involves a mammary hyper-response including lactogenesis and lactopoiesis and sometimes lactation. There is a mid-late luteal phase increase in prolactin-dependent stimulation of mammary tissues mimicking that of pregnancy. The incidence of some degree of ill-defined overt pseudopregnancy can reach 10–20% or more in some breeds, but is almost negligible in others (e.g. beagles). Precipitated by a premature decline in progesterone that either stimulates prolactin release or increases mammary prolactin-responsiveness seems likely in many cases. Mean prolactin is higher and mean progesterone lower in affected cycles vs. normal cycles. During the luteal phase ovarioectomy often precipitates an overt pseudopregnancy, and ovarioectomy, PGF induced luteolysis or antiprogestin administration can each increase prolactin. Clinical pseudopregnancy is often accompanied by, and is sometimes primarily manifested in, behavioral changes mimicking those of the prepartum bitch (e.g. circling, digging, nesting, and defensive behaviors). Clinical management is by prolactin-lowering
doses of dopamine agonists. Progestin administration is contraindicated as subsequent progestin withdrawal results in reoccurrence of symptoms. Terminology is confused because the long luteal phase of normal cycles has been referred to as “physiological pseudopregnancy” and analogous to those in rodents.

**Pseudopregnancy:**
Most, if not all, bitches show some evidence of pseudopregnancy during metoestrus, the intensity and signs being very variable; for this reason it is preferable to refer to *covert* pseudopregnancy, where the bitch will be in metoestrus but will show little or no signs, and *overt* pseudopregnancy. In the latter, the clinical signs will range from slight mammary development and lactogenesis whilst at the opposite extreme the bitch will show all the external signs of pregnancy and will ultimately undergo a mock parturition, with nesting, loss of appetite, straining, emotional attachment to inanimate objects and heavy lactation. Pseudopregnancy was originally believed to be due to an intensification and prolongation of metoestrus; however, a number of workers have demonstrated that there is no difference in the progesterone concentrations in the peripheral blood of bitches with or without signs of pseudopregnancy. It is likely that the prolactin may well be responsible for initiating the changes. If bitches undergo ovarohysterectomy when they are pseudopregnant the condition can be intensified and prolonged. Furthermore, antiprolactin drugs such as bromocriptine and cabergoline have been successfully used to cause remission of the signs of pseudopregnancy.

**THE CAT:**
Free-living non-pedigree and feral cats are seasonally polyoestrus, with a period of anoestrus beginning in the late autumn. Increasing daylight length is the most important factor in inducing the resumption of reproductive activity and the first oestrus will usually be seen soon after the shortest day of the year. If a constant 14 hours of lighting is provided daily, sexual activity will continue throughout the year, and this manipulation of photoperiod will alter the circadian rhythm of melatonin production. If the lighting regimen is changed from 14 to 8 hours then cyclic activity will cease immediately. There may be a period of apparent lack of oestrous activity in the early summer, but this corresponds with the pregnancy or lactation following mating earlier in the year rather than true anoestrus. Some non-pedigree cats have regular oestrous cycles lasting approximately 3 weeks, but others may show no regular pattern. The duration of oestrus is 7–10 days, and is not significantly shortened by mating. Oestrous cycle patterns show considerably more variation in pedigree cats. Long-hairs may have only one or two oestrous cycles each year, whilst the period of oestrus may be longer in Oriental queens with a reduced interoestral interval. Oestrogen concentrations increase dramatically at the time of oestrus from the baseline of 60 pmol/l, and may double within 24 hours, reaching a peak of up to 300 pmol/l. The principal oestrogen produced by the ovary is oestradiol-17β. The rapid rise in oestrogen concentrations corresponds to an abrupt appearance of behavioural changes indicative of oestrus, and queens do not usually show a distinctive pro-oestrous phase. The oestral display is characterised by increased vocalisation, rubbing and rolling. The queen becomes generally more active, and she will solicit the attention of a tom. The cat is an induced ovulator, and thus mating is important in triggering ovulation. Receptors are present within the queen’s vulva, which are stimulated during copulation, ultimately resulting in release of LH from the anterior pituitary. Only about 50% of queens will ovulate after a single mating, and multiple
matings may be required to ensure adequate release of LH to induce ovulation. The ovulatory surge of LH begins within minutes of coitus, peaks within 2 hours and returns to basal values within about 8 hours; peak LH concentrations of over 90 ng/ml have been reported. Further matings before the peak of LH concentrations has been reached will lead to additional increments. However, after multiple matings over a period of 4 hours or more, further matings may fail to induce any additional increase in LH concentrations, and this is thought to result from depletion of the pituitary pool of the hormone or development of refractoriness to GnRH. Ovulation is an ‘all or nothing’ phenomenon, and once significant concentrations of LH have been achieved all ripe follicles will rupture. The mean ovulatory rate for non-pedigree cats is approximately four, but is more variable in pedigree animals. Receptors similar to those found in the vulva are also located in the lumbar area, and these may be stimulated if the queen is mounted by other females or castrated male cats.

**Pseudopregnancy:**
Sterile matings, which successfully induce ovulation, lead to pseudopregnancy. Concentrations of progesterone are very similar to those of pregnancy for the first 3 weeks, after which levels gradually fall, reaching baseline by approximately 7 weeks. Nesting behaviour and milk production are rarely seen in pseudopregnant queens, but hyperaemia of the nipples will usually be evident as in pregnancy. The queen’s appetite may increase, with some redistribution of fat leading to an increase in abdominal size.

**ARTIFICIAL CONTROL OF CYCLICAL REPRODUCTIVE ACTIVITY:**
Control of the oestrous cycle for many years, since they would offer several management advantages:
- Successful methods of oestrous cycle control would facilitate the use of artificial insemination (AI) thereby allowing the greater exploitation of genetically superior sires. At present AI is underutilized particularly in beef cattle.
- AI could be carried out at a prearranged or fixed time if the control method was sufficiently reliable. This could potentially remove the need for the detection of oestrus. However, as will be seen later, the optimum performance is achieved when control of the cycle is used in conjunction with oestrus detection. In fact it may be said that control of the cycle serves to improve the rate of oestrus detection by concentrating the occurrence of induced heats into a shorter finite period of time.
- Synchronization of oestrus would allow the batch management of inseminations and calvings which in some circumstances would improve the efficiency of management. This is particularly true in groups of replacement heifers and in beef suckler cows, which are usually managed in groups and do not always receive the frequent and individual attention accorded to dairy cows. Under traditional conditions heifers and suckler cows are served naturally, due in part to the practical difficulties associated with the detection of oestrus. Therefore the use of pharmacological agents to control the oestrous cycle could potentially increase the use of AI in these situations.
- Synchronization may offer some advantages in shortening the calving to conception interval, and thus the calving interval and possibly the calving season, particularly in beef herds.

There are, however, a number of real or potential disadvantages to synchronization of ovulation:
- Currently, response to treatment and subsequent fertility are highly variable. Results vary considerably between herds and between years in the same herd. Consequently, the economic factors are variable also, but if reproductive performance is poor there will obviously be a low or negative cost benefit.
• There are often unseen costs such as the necessity for an increased labour input as compared to a natural mating system, in that the cows have to be collected and handled more frequently.
• Inseminators may tire and become less efficient if too many animals are to be served on one day.
• Adverse factors on any one day can affect a large number of animals’ ability to conceive, rather than just one or two.
• If successful, synchronization of a large batch of animals may result in excessive demands on labour and facilities at and just after calving.

**Principles and methods of cycle control:**
There are three main approaches:
• The artificial induction of premature luteolysis using luteolytic agents such as prostaglandin F2a (PGF2a). This will obviously only be effective in cycling cows with an active corpus luteum.
• Prostaglandin-induced luteolysis in association with GnRH to manipulate follicular and luteal function. This procedure could potentially be used for the induction of ovulation in acyclic as well as cyclic cows.
• The simulation of corpus luteum function, by administration of progesterone (or one of its synthetic derivatives) for a number of days, followed by abrupt withdrawal. This procedure is also effective for the induction of ovulation in acyclic cows.

**The artificial induction of premature luteolysis**
The most potent luteolytic agents available are derivatives of PGF2a. Injection of exogenous PGF2a or one of its analogues during the mid-luteal phase of the cycle results in premature luteolysis and a concomitant fall in peripheral progesterone concentrations. This is followed by a surge in secretion of gonadotrophins and estradiol-17b, culminating in the pre-ovulatory surges and eventual ovulation. The fall in progesterone concentrations is rapid, invariably reaching basal levels within 30 hours of injection. The time of luteolysis after the injection can be quite variable, depending on whether there happens to be a dominant follicle present at the time of luteolysis. This will depend on the stage of the cycle and whether the cow is undergoing a two- or three-wave cycle. The molecular structure of PGF2a and some of its analogues, which are available commercially for oestrus synchronization. Prostaglandins have been used to control the oestrous cycle in several different ways. Some possible methods are:
* Following rectal examination so that only those cows with a corpus luteum are injected. These cows should then show oestrus and ovulate 3–5 days later. This method has the disadvantage that it is time-consuming and that rectal palpation involves added expense. The results also depend on the accuracy of the rectal palpation.
* Following the identification of an active corpus luteum using milk progesterone measurement. A further milk sample could be taken before intended AI in order to confirm that the prostaglandin injection had induced luteolysis.
* Observation of all cattle for oestrus for a seven-day period, serving any that show oestrus. The rest are injected with prostaglandin on the following day and may be inseminated either once or twice at fixed times or at observed oestrus. The reason for the initial seven-day observation period is that there is a period of about seven days in the cycle (day 18 to day 0 and day 1 to day 4) when the animal is unresponsive to prostaglandin, i.e. when no corpus luteum is present. After seven days, those originally between days 18 and 0 should have shown heat and been served, while
those that were between days 1 and 4 will now be between days 8 and 11, i.e. in the mid-luteal phase, and therefore responsive to prostaglandin.

- The two injection plus two insemination method. The so-called two plus two technique was designed to synchronize groups of animals cycling at random without prior knowledge of their precise ovarian status. All cattle are injected on day 1 of treatment and the injection repeated 11 days later. AI is then carried out usually three and four days later. Alternatively, cows may be served at observed oestrus after the second injection. At the time of the first injection some animals will be responsive to the prostaglandin, i.e. between days 5 and 17 of the cycle. These will undergo luteolysis in response to the injection and will ovulate some four days or so later. At the time of the second injection (11 days later) these cows will be on about day 8 of the next cycle. The cows that were not responsive to the first injection, i.e. those between days 18 and 4 of the cycle, would be between days 8 and 15 at the time of the second injection. Therefore all animals are theoretically in the responsive mid-luteal phase at the time of the second injection. The technique is popular and quite successful in synchronizing cycles in heifers. However, pregnancy rates in lactating cows have not always been consistent and reasons for this are discussed later.

- A modification of the ‘two plus two’ method is the so-called 11/7 method. Cows are injected with prostaglandin and those that show oestrus are inseminated. Those that have not been seen in oestrus are injected again 11 days after the first injection and may be inseminated either at a fixed time(s) or at observed oestrus. Although requiring further effort in terms of estrus detection, this method tends to give better results than the ‘two plus two’ regime and is perhaps the current method of choice. Its main advantage, however, is the reduction in cost by the reduction of both the number of treatments used and number of inseminations per cow.

- The use of GnRH in conjunction with prostaglandin. The so-called Ovsynch regimen (Pursley et al., 1997) was designed to reduce the variability in the time of ovulation following the use of prostaglandin alone. GnRH is injected on day 0, followed by prostaglandin on day 7 and a further GnRH injection on day 9–10. Fixed-time AI is performed 16 hours later. The first GnRH injection is designed to either (1) manipulate ovarian follicular development by ovulating and/or luteinizing the existing dominant follicle and initiating the growth of a new cohort of follicles so that a new dominant follicle emerges by day 7, or (2) extend the life of the existing corpus luteum in late-luteal phase cows so that it is still responsive to prostaglandin 7 days later. The second GnRH injection is designed to synchronize ovulation further by initiating the pre-ovulatory LH surge, which should initiate ovulation. Peters et al. (1999a,b) found that ‘Ovsynch’, with the second GnRH being given on day 9.5, was effective and suggested that the major role of the first injection appeared to be the extension of the cycle in late luteal phase cows and that the second GnRH injection was the most critical in determining the synchrony of ovulation.
The simulation of corpus luteum function using progesterone:

In this method the function of the corpus luteum is simulated by the administration of progesterone or one of its derivatives. The progesterone suppresses gonadotrophin release, and hence follicular maturation, until it is withdrawn. If a group of cows is treated with progesterone and then it is withdrawn from all cows simultaneously, this will theoretically synchronize ovulation in the group. In order to synchronize a group of randomly cycling cows effectively, it is necessary to treat them with progesterone for a period equivalent to the length of the natural luteal phase, i.e. at least 16 days. This is due to the fact that exogenous progesterone has little or no effect on the life span of the natural corpus luteum and therefore in some cases the natural corpus luteum might outlast a short-term progesterone treatment, resulting in a failure of synchrony. However, it has been shown that long-term progesterone treatments (18–21 days) result in poor pregnancy rates. This is due at least in part to the ovulation of persistent follicles that ovulate oocytes of reduced fertility and possibly to adverse changes in the intra-uterine environment, which inhibit sperm transport. Shorter-term progesterone treatments (7–12 days) generally result in more acceptable pregnancy rates, but unfortunately tend not to control the cycle adequately since, if treatment is started early in the cycle, the natural corpus luteum may outlast the progesterone treatment. Therefore it is necessary to incorporate a luteolytic agent with short-term progesterone treatments in order to eliminate any natural corpus luteum. For this reason, oestradiol and/or a luteolysin is used in combination with short-term (7–12 days) progesterone treatments. Implants are the most suitable method of administration of progestogens since withdrawal can then be precisely controlled by implant removal. They can be inserted into the vagina or under the skin, usually in the ear.

Implants for insertion in the vagina include the progesterone-releasing intravaginal device (PRID; Sanofi Ltd.), which consists of a stainless-steel coil covered by a layer of grey inert silastic (Fig.) in which 1.55 mg progesterone is impregnated. A red gelatin capsule containing 10 mg oestradiol benzoate is attached to the inner surface of the coil. The PRID is inserted into the vagina by means of a speculum and is left in place for up to 12 days. The oestradiol benzoate in the gelatin capsule is rapidly absorbed through the vaginal wall into the systemic circulation and is intended to act as a luteolytic agent. The progesterone is released continuously from the elastomer until removal of the device. Removal is effected by pulling on the string which is left protruding from the vulva after coil insertion. PRID contains natural progesterone and therefore its effects can be monitored by measuring progesterone concentrations in the blood plasma or milk of the animal. A less bulky alternative (which could thus potentially be preferable for heifers) is the bovine controlled internal drug releaser (CIDR; DEC-InterAg). Originally, an oestradiol capsule was incorporated for luteolysis, but an intra-muscular (i.m.) injection on the day of insertion is now the preferred option. The advantage from using an i.m. injection of oestradiol on the day of insertion (instead of a capsule) and a further oestradiol
injection 48 hours after CIDR withdrawal to control follicular development. This should increase the precision of oestrus onset and the intensity of oestrous signs as well as more tightly controlling the timing of the LH surge and thus ovulation, so that the timing of AI can be optimized.

The other approach is to use an impregnated silastic subcutaneous implant (Crestar; Synchromate B; Intervet). The active ingredient is Norgestomet (17a-acetoxo-11b-methyl-19-nor-preg-4-ene,20-dione), a synthetic analogue of progesterone. The implant is inserted subcutaneously behind the ear for a period of nine days during which time the progesterone is absorbed into the blood circulation. The implant is removed by making a small scalpel incision in the skin of the ear over the implant. At the time of the implantation an intramuscular injection of 5 mg oestradiol valerate is given as a luteotropin, in combination with an initial injection of 3 mg Norgestrom. Removal of the device after 7–12 days causes peripheral plasma progesterone concentrations to fall, thus simulating natural luteolysis. Consequently, the cow should show estrus 48–72 hours later and fixed-time AI may be used at these times. This product is suitable for use in heifers so that there is no problem of withdrawing milk from sale during treatment.

**Physiological problems in cycle control:**

- The degree of synchrony following treatment. That is, the proportion of animals beginning to show oestrus or ovulating within a specified time period after the end of hormonal treatment.

- Subsequent reproductive performance (e.g., pregnancy rate), which will also to an extent be dependent on the degree of synchrony, particularly if fixed time AI is used.

**In the case of seasonal breeders** the ability to produce offspring out of season or to advance the time of onset of cyclic activity has advantages. In these and in other species, the ability to ensure that an individual or group of animals does not come into oestrus, or is in oestrus at the same time, has attractions. The methods that are available can be divided into two main groups: firstly, those which do not involve the use of hormones and, secondly, those that do.

**Non-hormonal methods:**

1. **Light**

The onset of cyclical activity in the mare, ewe, goat and cat is dependent upon changes in the hours of daylight. The mare and queen are stimulated to activity by a lengthening photoperiod, whilst in the ewe and goat it is the effect of a decreasing photoperiod which is the stimulus. In ewes, the provision of housing with controlled lighting enables the breeding season to change from the autumn and winter, to spring and summer. Furthermore, by subjecting the ewes to a lighting regimen which does not have any change in duration it is possible to ensure breeding throughout the year, as is the case in equatorial climates. If mares are stabled at the end of December in the northern hemisphere, and are subjected to artificial light, preferably of increasing duration, then it is possible to advance the onset of normal cyclical activity so that there is oestrus and ovulation. Both tungsten and fluorescent lights have been used, although the former would appear to be better. The provision of a 200 watt incandescent bulb in each loose-box is adequate, and if it is controlled by an automatic timing device, so that the duration of lighting is increased by 25–30 minutes each week, reproductive activity will be initiated when the mare is receiving 15–16 hours of light each day.

2. **Nutrition**

The effect of nutrition in initiating reproductive activity in seasonally breeding species is not clear. There is some evidence that the stabling of mares and the
provision of good feeding assist in stimulating the onset of cyclic activity in early spring. There is also evidence for the converse, when yarded mares are turned out to fresh spring grass about 80% of them have come into oestrus and ovulated within 14 days. Furthermore, he has found that barren and maiden mares maintained in yards on adequate but mainly dried feedstuffs during the winter and spring remain in anoestrus longer than those which are kept out at grass. An explanation for this is difficult to find, although it may be related to the β-carotene content of the diet, fresh spring grass containing large amounts of this substance. Improved nutrition can exert a profound effect on ovarian function by increasing the number of follicles which mature and ovulate. This effect is described as ‘flushing’, a practice which has been used in lowland flocks of sheep for many years. By increasing the dietary intake, particularly that of energy, before ewes are tupped it is possible to increase the number of lambs that are born. A similar technique can also be used in the sow to increase litter size. There is no evidence, however, that, provided the ewes are adequately fed, it is possible to advance the onset of the breeding season by this means. The opinions on the effect of nutrition on reproduction in the sow are conflicting. It is generally assumed that flushing gilts and sows 4–6 days before oestrus increases prolificacy by increasing ovulation rate. Whether this effect occurs in adequately fed individuals is difficult to determine.

3- Other non-hormonal methods:
The presence of a male animal can exert its effect upon the cyclical activity of the female. This is well demonstrated in sheep, where the introduction of a vasectomised tup at the start of the breeding season will stimulate the onset of oestrous cycles in the majority of ewes and can also bring about some degree of synchronization of cyclical patterns.

Hormonal methods:
Mare: In some racehorses and show-jumpers it is desirable to prevent the mare from coming into oestrus at an inappropriate time; in some cases it may be desirable to synchronise a group of animals. A daily injection of progesterone at a dose rate of 0.3 mg/kg is effective in preventing oestrus, with a return to a normal fertile heat 3–7 days after treatment ceases. Potent oral progestogens are now also available for suppressing oestrus and synchronizing groups of mares when withdrawn. One of these, allyltrenbolone or altrenogest, has been used successfully in a number of ways:
  • To stimulate the onset of cyclical activity. A good response will be obtained when given in the late transitional phase from anoestrus to cyclical activity when follicles are present. The results are better if increased lighting is used.
  • To suppress oestrus – for example, for shows or other functions.
  • To suppress oestrus in mares with prolonged oestrus or other aberrant sexual behaviour.
  • To control the time of oestrus so that effective use can be made of a stallion or artificial insemination. The hormone should be fed for 15 days and then stopped, so that the mare should come into oestrus 2–3 days later. Prostaglandins, both PGF2α and the synthetic analogue cloprostenol, are useful in the
breeding management of mares. By enabling mares to be mated on predetermined days it is particularly useful where either the mare or the stallion has to travel a distance for service, and eliminates the need for the frequent teasing of mares. It is also useful if a heat is missed, especially the foal heat, since it enables oestrus to be induced prematurely and obviates the need to wait for the next spontaneous heat.

**Ewe:**
Progestogens have been widely used in controlling reproduction in the ewe, either on their own or in conjunction with other hormones. They have been used to induce oestrus in the anoestrous ewe during the non-breeding season, and also for synchronisation of groups of ewes that are already showing cyclical activity. Most of the progestational substances are administered via the intravaginal route in the form of impregnated sponges or tampons. Provided that the progestogen is correctly incorporated into the sponge, it is readily absorbed at a sufficient rate to ensure a full negative feedback effect on pituitary function. When intravaginal sponges are used outside the normal breeding season, it is necessary to use eCG as a source of gonadotrophin at the end of the progestosterone priming period. The dose of eCG required is such that it should stimulate oestrus and ovulation without causing superovulation. Opinions vary on the time of injection of eCG. Whilst it is claimed that better results are obtained if it is injected 48 hours before sponge removal, the advantage is so small that the additional handling of the ewes does not make it cost-effective. When PGF2α or an analogue is given to ewes with sensitive CLs, oestrus occurs 36–46 hours after injection. In order to synchronise a group of ewes at randomly different stages of the oestrous cycle it is necessary to give two injections 8 or 9 days apart. Conception rates and prolificacy following natural matings have been comparable with unsynchronised ewes. These are obvious advantages of using such a technique in conjunction with AI since it could enable the use of genetically superior sires in many flocks.

**Melatonin:**
The pineal gland controls reproductive activity in seasonal breeding species such as sheep, goats, horses and cats by the secretion of melatonin. Perhaps not surprisingly, it cannot be used to modify seasonal activity in the mare because it would be necessary to inhibit the secretion of melatonin or neutralise its effect to advance the time of onset. However, in the ewe and doe, which are short-day breeders, it has been used commercially to advance the timing of the onset of the breeding season. The hormone is administered as an implant containing 18 mg of melatonin, which is inserted subcutaneously at the base of the ear. It is critical that rams (and bucks) should be separated from the ewes so that they are out of sight, sound and smell at least 7 days before the insertion of the implant. They must remain separated for at least 30 days and not more than 40 days, when rams (or bucks) should then be reintroduced. Peak mating activity occurs 25–35 days later. Melatonin should not be used in ewe lambs. The breeding season can be successfully advanced by 2–3 months with good fertility.

**Immunisation procedures:**
Increased lambing rates have been obtained by the use of an immunogen, produced by conjugating a derivative of the natural ovarian hormone androstenedione with human serum albumen. When injected into ewes the conjugate stimulates the production of antibodies to androstenedione, which binds free, naturally occurring androstenedione in the blood. This results in an increase in the ovulation rate and the number of lambs born; the precise mode of action is not fully understood. The conjugant is injected
twice, 8 and 4 weeks before tupping, although if ewes have been treated in the previous season one injection only is required (4 weeks before tupping). The effect of immunization is to increase the lambing percentage by about 25%. It is important that only those ewes which are likely to be fed adequately during pregnancy should be treated because of the dangers of pregnancy toxaemia; for this reason, mountain and hill breeds should not be treated. Immunisation against inhibin, which has been used experimentally to increase the ovulation rate in cattle and sheep, may well become available for commercial use.

**In the Mare:**
**ONSET OF THE BREEDING SEASON**

A conflict often arises between horse breeders and mother nature. This is because we usually wish to breed horses at the earliest limit of their breeding season, at a time when they are least fertile. In 1833, the British Jockey Club ruled that all foals should take their birthdays from January 1st so that no horse would change its age during the racing season.

In the southern hemisphere, similar impositions on reproductive physiology exist. The official stud book birthday for the Thoroughbreds in Australia is Aug 1st. This is only slightly more congruent with reproductive physiology than their official birth date to the north. However in true Australian style, Standard breed breeders have (in the words of an Australian colleague) “thumbed their noses at the establishment” and have set the official birthday for Standard breeds as Sept 1st, the most physiological yet.

**Management reasons for early season breeding**

Early season breeding is also practiced for management reasons. For example, some horse owners prefer early foals that can be weaned at three or four months of age, making mares available for riding or competition during the summer. Furthermore, early foals are important to breeders because weanlings and yearlings are more impressive in the sales ring, commanding higher prices.

**Why some breeders do not breed early in the season**

Although it has been stated that two year olds which foaled early will perform better than their counterparts at any given time, interesting arguments can be made against this statement. For example, some information shows that significant differences between early and late foals may only exist during the first year of life and that late season births are followed by unexplained rapid growth, allowing all foals to be similar in size by two years of age. Anecdotal statements substantiate this by horse owners who point out that many superior racehorses are the product of late breeding season. Finally, one should also bear in mind that some horse owners do not like to breed their mares early in the season because foals that are born early are confined to barns in cold weather. In these situations, population pressure is often high and the threat of disease is a greater than when foals are at pasture. For those reasons, no one practices early season breeding. In situations where early season breeding does occur, a constant contra-physiological struggle also occurs, because during springtime, mares experience prolonged estrous periods and often fail to ovulate. This time is known as the transitional period.

**USING LIGHTS TO ‘AVOID’ THE TRANSITIONAL PERIOD**

It is well known that light treatment can be used to hasten the onset of the breeding season (by 10 to 12 weeks). However, light treatment will not hasten the onset of the breeding season; it will not eliminate the transitional period. The prolonged estrous periods and failure of ovulation that are typical of transitional mares, will still occur.
Therefore, the advantage of using lights is to ensure that the transitional period has passed before early season breeding begins. Because the problems of the transitional phase also occur when lights are used to start early season breeding, several hormone treatments have been tried to circumvent them. That is the subject of the next section.

HORMONAL ATTEMPTS TO CURTAIL THE TRANSITIONAL PERIOD

Using GnRH

As expected, a good deal of attention has also been given to the potential of gonadotrophin releasing hormone (GnRH) in the context of early season breeding. GnRH is a hypothalamic peptide hormone that causes the release of LH and FSH from the anterior pituitary gland (adenohypophysis). The physiology of GnRH secretion and attempts at using the native hormone and its analogs to induce early season estrous cycles have been reviewed elsewhere.3 Treatments have included single and multiple injections of GnRH or its analogs, chronic infusions, and the use of GnRH in miniature osmotic pumps. Despite promise in some respects, GnRH treatments have generally been ineffective, expensive or impractical. Absorbable, subcutaneous implants containing a potent GnRH agonist analog called deslorelin ie. “Ovuplant.” can produce sustained LH release in mares and has shown promise in inducing early season cycles. However, up to six repeated implants may be needed (q.48 to 72h) to induce cyclicity so it will probably not be used widely for this purpose. However a single implant of deslorelin together with progestogenic priming may hold some promise for inducing early season cyclicity.

It should be noted that all GnRH treatments have the potential ability to down regulate the hypothalamic pituitary axis causing gonadotrophic secretion, a phenomenon well documented in other species but only recently noticed in horses.6b The value of progestogenic priming to curtail transition is discussed later in this paper.

Using FSH, LH, pituitary extracts & hCG

FSH is not usually the limiting hormone with regard to the seasonal onset of estrous cycles. Although a relative absence of both FSH and LH limit initial ovarian activity, serum FSH concentrations rise at least two months before the beginning of the breeding season and there is vigorous follicle growth long before ovulation occurs. By contrast, serum LH concentrations remain low until just before the first ovulation of the season. Therefore there appears to be less need to amplify FSH secretion than LH secretion to promote cyclicity.

Attempts have been made to hasten the onset of estrous cycles by crude replacement of both FSH and LH. Ovulations can be induced in anestrous mares by multiple injections of equine pituitary extracts (porcine FSH is not very effective in mares) but these extracts are expensive and difficult to make. Small amounts are available for research but generally equine pituitary extracts are unavailable. They usually contain both FSH and LH, and when used in anestrous mares, these extracts can induce ovulations. Some mares have become pregnant when bred to these ovulations while others returned to seasonal anestrus. Human chorionic gonadotrophin (hCG) has also been used for its LH-like activity during the early transitional period but in one study, multiple injections of hCG given at low doses (200 IU per dose) had no significant effect on ovarian function. However, later in the transitional period, the treatment was successful although some of the mares that ovulated then relapsed into seasonal anestrous (like those treated with pituitary extracts). Treatment also induced high
titres of antibodies against hCG, potentially limiting the value of hCG treatments later in the breeding season. In clinical practice, many veterinarians will have noticed that injections of hCG frequently fail to induce ovulation early in the breeding season, even if very high doses are used. This is substantiated by research findings as well. The reason for this is unknown but it seems rational to suggest that there is a deficiency of LH receptor sites in follicles at that time of year. Therefore, when confronted with ovulation failure, one should not resort to multiple injections of hCG because, as mentioned previously, antibodies against hCG may be produced. Although these antibodies last for several months they do not cross-react with the endogenous LH of the mare11 as they will in primates.17 Therefore even if hCG is abused, mares will continue to cycle and ovulate normally.

**Using eFSH**

Although there is a basic shortage of LH during transition, there is probably a shortage of FSH as well despite the presence of multiple small follicles in the ovaries. In that regard, it has recently been shown that FSH supplementation in transitional mares using equine pituitary extracts (eFSH Bioniche™) will shorten transition when compared to non-treated animals. In these treatments, mares in transition (FSH was not effective in mares with completely inactive ovaries) were given 12.5 mg of eFSH twice daily for a maximum of 15 consecutive days. When the largest follicle was at least 35 mm in diameter, 2,500 i.u. of hCG was given and the mare was bred. The percentage of mares ovulating within that 15 day period was 80% compared to 0% for untreated mares. Treatment induced ovulation at a mean interval of 8 days instead of 40 days. Only recently has this treatment become feasible because until 2003, eFSH has not been commercially available. Even now, it is only available in limited quantities; small amounts are extracted from large numbers of slaughtered horses.

**Using progestogens**

Progestogen (progestosterone-like hormones) treatments can hasten the first ovulation of the year18-23 by decreasing the incidence of prolonged estrus and anovulation. Progestogens will not make the transitional period occur earlier than usual; as is the case with lighting, but appear to “de-fibrillate” the hypothalamic-pituitary-ovarian axis during transition. The use of the synthetic progestogen altrenogest illustrates this principle well. When mares were treated with altrenogest for 15 days during the transitional period, estrous cycles were more regular after treatment was stopped than if no treatment was used. However, follicles were smaller and serum estrogen and LH concentrations lower, than at the same events later in the year. Also, estrus was usually longer after altrenogest treatment and the time of ovulation was later relative to the beginning of estrus than in normal cycles. In other words, the characteristics of transition were not suppressed entirely.

Mares can certainly conceive during these early season, post-progestogen estrous cycles, therefore altrenogest or progesterone are frequently used in combination with lighting programs for early season breeding.

The mechanism of action of progestogens during the transitional phase is not known, but it seems reasonable to suggest that they prime behavioral centers in the brain and also suppress GnRH secretion, allowing a rebound of LH secretion when treatment is stopped. Whatever the mechanism, it is clear from data on this subject that mares must be well into transition to be able to respond to treatment. In other words, progestogenic treatments are only effective in mares that are on the verge of having estrous cycles anyway. Consequently, progesterone or altrenogest treatment should only be used if mares are actually in a state of estrus or have follicles larger than
20mm in diameter. Progesterone is much less expensive than altrenogest but it must be injected every day and is therefore far less convenient than altrenogest, which is given orally. Although no comparisons have been published, a combination of progesterone and estrogen is probably more efficient than progesterone or progestogens alone for these treating transitional mares. In a group of 200 mares treated with light then progesterone and estradiol, there was an 80 percent increase in the number of mares in foal at the end of February compared with control mares only treated with light. The progesterone and estradiol treatment typically used is the same as described elsewhere for estrous synchronization. However, it should be emphasized that good control over the time of ovulation (characteristic of the use of “P&E” in cycling mares) cannot be expected when this treatment is used in transitional mares!

**HORMONAL TREATMENTS TO USE WHEN ESTROUS CYCLES ARE ESTABLISHED**

One may wish to ensure that a mare is in estrus and ovulates at a pre-appointed time so that she can be examined, inseminated or even used as a mount mare for semen collection or for stallion evaluation. Controlling the time of ovulation is particularly useful for broodmare owners that do not have a stallion to tease with or have no experience or facilities for teasing. Estrus and ovulation can be scheduled on demand. Also, when artificial insemination (AI) is used, one can limit the number of times that semen is collected, packaged and shipped, all costly procedures. Even if AI is not used, a mare’s stay at a stud farm can be shortened to one or two days instead of several weeks. Controlling the time of ovulation is also extremely important when frozen semen is used because the time between ovulation and insemination is critical. Ovulation can also be synchronized in groups to facilitate breeding and of course, synchronized ovulations are essential for embryo transfer (ET).

To understand estrous synchronization, one must bear in mind that there are two essential elements in the process: i. Control of the luteal phase and ii. Control of follicle growth. An absence of luteal tissue and the presence of a mature follicle are essential if ovulation is to occur at a pre-determined time.

In ruminants, where estrous synchronization is widely practiced, follicles mature every 4 or 5 days, therefore large follicles are almost always available for ovulation. As a result, control of follicle growth is not as critical as in mares. Even in basic progestogen or prostaglandin treatments in cattle, where there is essentially no control over follicle growth, follicles in various stages of growth are recruited rapidly after treatments and ovulations, although distributed over a few days, are still quite synchronous.

In contrast to cattle, most mares only have one follicle wave during an estrous cycle and unless treatment includes a hormone to control follicle growth, the time of ovulation will not be controlled when treatment ends. In fact, because follicle growth and maturation occurs over a period as long as 18 days (the exact period is unknown) ovulations can occur over a protracted period of time after treatment ends. This is why progesterone or altrenogest treatments alone are poor methods for synchronizing estrus in mares. It also explains why inter-ovulatory intervals in mares cannot be shortened consistently with prostaglandins (see section on “short cycling” in mares).

**PROSTAGLANDINS**

**General luteolysis**

Prostaglandin F₂α (PGF₂α) and many of its analogs have been used in horses. The generic names and common doses of these hormones are mentioned in the following table. Approval for use in horses varies from country to country. As most of these
analogs are available in the USA, the approval status in that country is given. Native PGF2alpha and its analogs may be administered by any parenteral route but subcutaneous or intramuscular injections are usually recommended. Early work with PGF2alpha showed that no particular route of administration was more effective than another and that intrauterine administration held no advantage over parenteral administration. 31,32 The latter finding is distinctly different from the case in cows but it is not surprising because a local luteolytic pathway does not exist in mares.

Apart from the fact that large follicles are seldom “waiting in the wings” during the mid luteal phase in mares (see earlier) the equine luteal phase is very short in mares, the shortest of all domestic species! In fact, luteolysis is only reliable after exogenous prostaglandins for about 7 days in the whole estrous cycle. 31-35 Therefore prostaglandins have less control over the equine estrous cycle than is generally thought. What then creates the impression that prostaglandins work so well in mares? Probably the fact that normal mares actually spend almost half of their cycle either in estrus (6 days) or coming into estrus (3 or 4 days) without any prostaglandin treatment.

Using prostaglandins to control the cycle

This approach to estrous synchronization in mares was adapted directly from ruminants where luteal termination works well to synchronize estrous cycles. Luteolysis eliminates the suppressive effects of progesterone on estrous behavior and LH release. In ruminants, follicles may be large and ready to ovulate or at the most will take about five days to mature after luteolysis, so ovulations are quite well synchronized although they occur over several days. By contrast, in most mares, there is just one follicle wave and the time to follicle maturation is prolonged. Therefore, the stage of development of the follicle that dominates estrous synchronization in mares, not the time of luteolysis.

Although the interval to the time of ovulation after prostaglandin treatments is commonly given as “nine to ten days” this figure is practically useless because estrus and ovulation can occur almost immediately after prostaglandin treatment or ovulation can be delayed for more than two weeks.

Despite these limitations, there are numerous reports of prostaglandins having been used to “synchronize” estrous cycles of mares for embryo transfer or other reasons. 39-44. If two injections of prostaglandin are given 15 days apart approximately 90% of mares can be expected to show estrus by the sixth day after the second injection, and conception rates should be normal. However, these data need to be interpreted with caution: although the modal time of ovulation after this treatment is usually between seven and ten days, ovulation can occur anywhere between day 0 and 17 days.

Even when hCG is given to decrease the time span over which ovulation occurs the time of ovulation is still not highly predictable.

The concept of synchronized cycles is relative in these studies on prostaglandin. Such synchronization treatments are clearly imprecise. If one merely needs to “group” the time of ovulation in a band of mares or if only one or two mares need be selected as E.T. recipients from a “synchronized” group, this degree of synchronization may suffice. However, most owners do not have large groups of mares from which they can select those mares most closely synchronized. In addition, when appointment breedings are to occur with shipped or frozen semen, ovulation must be more accurately controlled than this.

When prostaglandins appear to fail

Prostaglandin treatment may appear to fail when luteolysis occurs and large follicles are present in the ovaries. This is possible in the 25 to 30% of mares that do indeed
have two follicle waves in a cycle. If the follicle present during dioestrus is almost mature, the onset of estrus can be so rapid and estrus so abbreviated, that estrus and ovulation are not observed. In such cases luteolysis and ovulation are almost synchronous, with the formation of the new CL masking the lysis of the original CL, giving one the impression that luteolysis must has failed. Prostaglandin itself may also cause enough LH release to result in ovulation. Even before luteolysis has occurred! This phenomenon seems to be most likely when potent prostaglandin analogs are used. Therefore, as a precautionary measure, one should always determine if large follicles present before prostaglandins are given to induce luteolysis.

The concept of “short cycling” mares

This concept has been borrowed from bovine theriogenology but is not as effective in mares as it is in cattle. Short cycling is often touted as an adjunct for treating genital infections and is sometimes used to return mares to estrus as soon as possible if ovulations have been missed. However, because of the limitations already mentioned (short luteal phases & single follicle waves in most mares) “short cycling” treatment is usually ineffective. For example, in two controlled studies, the mean interovulatory interval was barely shortened (range 14 to 21 days) even though prostaglandins were given on the sixth to the eighth of the luteal phase. Estrous cycles will only be shortened significantly when a second (early diestrous) follicle wave is present.

PROGESTOGENS

General remarks

As an alternative to the use of prostaglandins, artificial luteal phases have been used in attempts to control estrous cycles of mares. Again, these treatments have been adapted from the cattle industry where they generally perform quite well. First, consider how they work in ruminants:

An artificial luteal phase (supplied in the form of oral, injectable, vaginal or subcutaneous progestogens) suppresses LH secretion and prevents the occurrence of estrus while treatment continues. With some variations, treatment is given for long enough to exceed the life span of any CL present. If shorter duration progestogen treatments are given, luteolytic treatment with prostaglandin is also given. In either case, no endogenous luteal tissue will be present in any animal when treatment ends. (If that were the case estrus and ovulation would not occur in such animals). When progestogen treatment ends, follicles mature and ovulate in all treated animals within 4 or 5 days of the end of treatment. This is because ruminants have such frequent follicle waves; usually three to four every 21 days. This provides satisfactory synchronization of both estrus and ovulation.

In mares, by contrast, progestogens do not work well for estrous synchronization. One reason for this is that the time of ovulation depends on the stage of follicle growth when treatment ends and because there is usually just one follicle wave, there is greater variation in the time taken to develop from immaturity to maturity than in ruminant. A second reason that progestogens do not work well for estrous synchronization is that they are not particularly effective at suppressing ovulation in mares. Evidence for this phenomenon is found in the fact that mares can ovulate spontaneously during the luteal phase and of course, they ovulate frequently during pregnancy (forming accessory corpora lutea) when serum progesterone concentrations are very high. Ovulation can also occur during treatment with the oral progestogen altenogest. The most common progestogens (progestins) used for controlling the estrous cycle of the mare are progesterone itself, and altenogest. Other progestogens have been used but few data are available on their efficacy. Most
data are available on the use of altrenogest, which is popular with horse owners because it is administered orally and is both safe and fairly effective at suppressing estrous. When altrenogest is used in mares showing estrus, estrous behavior usually stops within two or three days but in some cases, may persist for up to 6 days. In the author’s experience, altrenogest is unable to block estrous behavior in some mares, even at twice the recommended dosage (0.088 mg/kg vs. 0.044 mg/kg) suggesting that some mares may have an extraordinary ability to catabolize the hormone. After 15 days of altrenogest administration, most mares start to show estrus by 3 to 5 days and ovulation usually occurs 5 to 6 days after that i.e. 9 to 11 days after the end of treatment. However, because of reasons already discussed, ovulation can occur between day 1 and 15 days after treatment. Altrenogest is not effective for estrous control. Instead, the strengths of altrenogest lie in its ability to regulate “transitional” mares, suppress estrus in show animals, and maintain pregnancy and in its unique route of administration. Importantly, altrenogest does not cross react with most progesterone assays, allowing endogenous progesterone assay while altrenogest is being administered. The author finds the latter attribute especially useful when altrenogest is being given to support tenuous pregnancies e.g. after difficult twin crushes and when late gestation (>20 days) co-twin fluid aspiration is practiced. It can also be used when owners insist on “progesterone supplementation” in pregnant mares. In such cases, endogenous progesterone can be monitored intermittently to decide when or if to wean the mare off altrenogest treatment.

ESTRADIOL USED TOGETHER WITH PROGESTOGENS

Improved control over the estrous cycle can be obtained by using estradiol together with progesterone (“P & E”) or other progestogens. This is by far the most effective way to control the estrous cycle of mares. Treatment is started at any time of the cycle, with regard to nothing more than genital health and the fact that the mare is definitely having estrous cycles (the importance of the latter point cannot be overstated). After treatment, insemination by appointment on the ninth day after treatment ends will produce excellent fertility. The capability of P & E treatment rests mainly on the modest suppressive effect of estradiol on FSH secretion controlling follicle growth. The progesterone component of P & E treatment suppresses estrous behavior and to some extent ovulation. After ten days of treatment, estradiol produces a state where there are no follicles larger than about 2 cm. in diameter in the ovaries. Therefore, when treatment ends, all treated mares take about the same time to develop a pre-ovulatory follicle. Eight to ten days after P & E treatment ends, these follicles vary slightly in diameter they are all ready to ovulate. An injection of hCG on day eight (after the end of P & E treatment) effectively synchronizes ovulations so that they usually occur on day 9.5 days after P & E treatment ends. Although a combination of estradiol and progesterone may suppress LH secretion more than progesterone alone, it should come as no surprise (from comments already made about the ability of progestogens to control the estrous cycle) that ovulations also occur during P & E treatment. Most of these are from follicles that were present before treatment started. Because estradiol only has a mildly suppressive effect on FSH secretion in mares and large follicle may have already bound sufficient FSH to grow to a pre-ovulatory size, those follicles that are present at the onset of P & E treatment often ovulate during treatment. This is why prostaglandin is always given at the end P & E treatment; to lyse any pre-existing CLs and any CLs that may have formed during P & E treatment. It becomes apparent that some mares may even ovulate up to the last day of treatment. CLs formed after such ovulations would be too immature to undergo luteolysis after prostaglandin treatment of day 10 of P & E
treatment and with a 15 day life span, the progesterone from those CLs could interfere with ovulation on day 9.5 after the end of treatment. P & E treatment seldom fails to give excellent results but this phenomenon would explain some failures. Administration of prostaglandin on day 8 (when hCG is normally given) would prevent this problem. As mentioned earlier, an important reason for P & E failure is because mares are not yet cycling properly when treatment commences. We believe this is a significant problem in northern regions where mare may have their first ovulations as late as April or even May. It is essential to demonstrate that a mare is cycling before treatment starts. This can be done by demonstrating a CL on ultrasound, by a single elevated progesterone concentration (>4ng/ml) or by routine teasing, demonstrating clear aggression to a stallion after a period of estrus. The display of estrus alone is not sufficient as these mares are often in transition. P & E treatment failure can probably also occur when mares are under-dosed. For that reason, we suggest that the dose for draft mares be increased accordingly and that packaging should allow for wastage. Under dosage may also be a problem in cold weather because when P & E is kept in a cold barn, the steroids will precipitate out of the oil carrier and mares will be under-dosed. For that reason, P & E should be kept warm at all times. Those contemplating the use of P & E should see other notes on the subject (http://www.upei.ca/~lofstedt/opence/pande.pdf). P & E has been associated with normal to increased conception rates and is an extremely useful adjunct to stud management; P & E has also been incorporated into single injection of absorbable microspheres in a system known as “Lutamate plus” (Thornbrook farms, NY, NY) but precise control over ovulation was lacking. Nevertheless, the Lutamate plus system is convenient and innovative and could be used when less precise synchronization is desired than that possible with conventional P & E treatment. Because conception rates are marginally lower at foal heat and embryonal death rates higher than at subsequent estrous periods, attempts have been made to delay foal heat using P & E so that additional uterine involution can occur before breeding. These treatments hold promise but not enough data are available to substantiate their general use.

**INDUCING OVULATION DURING ESTRUS**

**The use of hCG**

For optimal fertility, ovulation should occur as close to the time of insemination as possible, especially when frozen semen is used. This is because freeze-thawing shortens the lifespan of spermatozoa. Also when shipped semen or natural breeding are used, additional cost, effort and time can be saved if ovulation occurs on demand. It is also very common to use induce ovulation as soon as possible after inseminations done on a Friday, the rationale being that the semen has to remain viable for the next three days (until Monday) and ovulation could occur late in that time period. This could theoretically compromise the chances of conception. hCG is the common hormone used for this purpose in horses. Readers should recall the cautionary statements made earlier in this article about the poor efficacy of hCG when it is used early in the breeding season and the possibility of provoking antibody formation through frequent injections. Single doses of between 2000 to 3000 i.u. of hCG given to cycling mares during the first two or three days of estrus have been shown to induce 80 percent of them to ovulate within 48 hours and will usually shorten the estrous period by one or more days. It has been reported that large single doses of hCG (4,500 to 6000 IU) could cause infertility but this has not been re-examined by other investigators. Recent data accumulated on a large number of mares shows that as little as 750IU of hCG S.C. is still effective in inducing ovulation in 80% of mares within 48 hours of injection.
The use of GnRH
One might expect GnRH to be an excellent substitute for hCG because of its ability to release LH but mostly negative results were obtained when native GnRH was used for this purpose. However, very high doses of native GnRH and repeated injections of potent GnRH agonists such as buserelin, leuprolide, or slow release formulations of deslorelin, can induce ovulation.
In the last few years, the GnRH analog deslorelin has been available in implant form ie. Ovuplant. Its potential use in inducing estrous cycles during late anestrus has already been discussed. In cycling mares, it is highly effective in inducing ovulations during estrus. Unlike hCG, deslorelin is a small molecule, similar to endogenous GnRH. Therefore repeated should not cause antibody formation as is the case with hCG. Deslorelin causes large amounts of LH to be released over a period of about 6 days after implantation and although the LH profile is different to that of an endogenous surge, ovulation is effectively induced within 48 hours by a 2.2 mg implant. As with hCG, mares should only treated when a follicle larger than 30 mm in diameter is present. Mares should not be implanted with larger doses of deslorelin than 2.2 mg and treatment should not be repeated frequently. Because deslorelin, like other potent or chronic GnRH treatments, tends to down-regulate FSH and LH secretion. At high doses, it can suppress follicle development, lengthen the estrous cycle or can even stop estrous cycles completely.
Deslorelin is also stable whereas re-constituted hCG must be used or discarded if not frozen immediately. Unfortunately deslorelin is expensive, about 5 times the cost of hCG. Use of GnRH analogs to increase pregnancy rates.
A recent report on more than 2000 mares showed that doses of the potent GnRH analog buserelin would improve pregnancy rates by as much as 105 when given between day 8 and 12 after breeding. These data have not be duplicated with other GnRH analogs.
SUPEROVULATION
Superovulation of mares is not nearly as effective as in other farm species but during the first five days of estrus, multiple injections of crude equine pituitary extracts given daily or commercial porcine pituitary FSH given twice daily produced between 1 and 4 ovulations occasionally more. It is unlikely that superovulation in mares will ever be as effective as in cows because of the physical restriction placed on multiple follicles growing by the thick tunica albuginea around the ovary and by the fact that all ovulations must pass through the ovulation fossa. Native GnRH has also been used in attempts to superovulate mares but even when massive doses were used, no superovulation was evident. In fact, from data already discussed on the use of superactive analogs, it appears that these treatments would be more likely to have the opposite effect to superovulation, down-regulating the release of gonadotrophins and causing ovarian inactivity. Recently, an equine FSH (eFSH) extract has become available, virtually doubling the chances of collecting two embryos per flush. Its availability from Bioniche Animal Health is limited. The mean ovulation rate and embryo rate using eFSH can be as high as six or seven oocytes with some treatments but the highest percentage of ovulations (about 3.9 on average) and embryos (1.9 on average) may be obtained using 12.5 mg eFSH q 12 h. Treatment are best when there are no dominant follicles (>25mm) in the ovaries at the time when treatment is started. This is usually at under day 6 of the cycle.
OXYTOCIN
Oxytocin is a small molecule, just nine amino acids, and is therefore fairly stable, easy to manufacture and inexpensive. Although it has a shelf life of about 2 years at
room temperature, its biological activity can degrade rapidly at higher temperatures. This author believes that he has seen the results of degraded oxytocin reflected in its inability to induce foaling in a mare. When a properly stored dose of oxytocin was administered, foaling ensued rapidly.

**Duration of action**

One should also bear in mind that oxytocin has a short circulating half life. In humans, it is in the order of a few minutes, being rapidly degraded by the liver and kidneys. In non-pregnant mares, it is about 7 minutes. A recent study using intra-uterine pressure transducers showed that a single bolus injections of 10 I.U. only increased uterine contraction for about 20 minutes. It also showed that as the dose (2.5,5 and 10 I.U.) increased, the frequency of contractions and duration of stimulated motility increased but the strength of contractions did not increase significantly. These data suggest that there may be little clinical value in increasing the dose of oxytocin beyond 20 I.U. for a 500 Kg mare. Some caution should be used in interpreting those data however, since intra-uterine pressure measurements may be unreliable, especially in comparison to electromyo graphic studies. Although higher doses may not increase the strength of contractions they definitely cause abdominal pain. In fact, single doses exceeding 50 I.U. can cause mild to sever colic. As the therapeutic value of such high doses is questionable, we generally suggest that doses should not exceed 20 I.U. Long acting analogs have been available for human use from some time but are only recently being examined in animal science. The most common of these, carbocetin, causes prolonged uterine contraction in comparison to native oxytocin in pigs102 and appears to be safe for use in mares as well. Unfortunately, this analog is not yet available to veterinarians in North America. Therefore, multiple injections must often be given to achieve a clinical effect. A notable exception to this statement is induction of foaling.

**Oxytocin for inducing foaling**

For numerous reasons (especially failure of passive transfer, premature placental separation and occasionally, dystocia) it is not wise to induce foaling routinely. Even if the cervix is softened with PGE2 with prior to induction, these problems are still likely to occur. This author used to induce foaling commonly for teaching purposes and other reasons but eventually had enough experiences with the drawbacks of induction to become convinced that it should not be used flippantly. Sometimes however, the procedure will be indicated, for example, when a mare has ventral abdominal rupture, severe injuries, or there is potential for neonatal iso-erythrolysis. These factors have been discussed and referenced elsewhere. In all cases, when doses of more than 20 to 60 I.U. are used, foaling is induced rapidly (within 90 minutes). Therefore there is no time for the foal’s lungs to mature and for the udder to develop as it does in cattle inductions. In addition, the procedure is essentially irreversible and the outcome is the direct and immediate responsibility of the attending veterinarian.

In conventional induction, single bolus injections of 20 to 30 I.U. of oxytocin are given intramuscularly. Alternatively, repeated injections of 15 IU at 15 minute intervals can be given. Oxytocin drips similar to those used in humans have also been described i.e. 1 I.U. per minute (to a maximum of 75 I.U.) in saline is given. Various methods of induction have not yet been studied in a single controlled trial. The progress of foaling should be monitored after the first five minutes via per vagina examinations and again throughout early second stage to prevent dystocia. As usual, one should also be alert to the possibilities of premature placental separation and asphyxiation by the amnion. Neonatal resuscitation is equally important.

**Foaling response to low-dose oxytocin in mares that are due to foal soon anyway**
One publication suggests that a dose of oxytocin as low as 2.5 I.U. can be used to induce foaling safely in mares. In 16 mares treated this way to induce daytime foaling, there were no differences in foaling health between the treatment and control groups. All the treated mares foaled during daytime whereas 58 of 60 control mares foaled at night. Most interesting however, was the finding that mares did not appear to respond to the treatment until a certain stage of feto-placental maturity was reached. This approach deserves further study.

**Oxytocin for retained placentas**

Retained placenta is commonly treated by repeated injections of oxytocin and occasionally by drips as described for induction of foaling. Both treatments are highly effective but the latter approach is used when mares are on intravenous drips anyway, for example after cesarean operations. Intravenous drips are impractical under farm conditions. The author is strongly inclined to leave a bottle of oxytocin with broodmare owners and to instruct them on its use (and storage). The usual recommendation is for the owner to start treatment when the placenta has been retained for longer than three hours and to administer 20 IU I.M. every 20 minutes for a maximum of six injections. This is also justified in the light of recent data showing that placentas retained for longer than four hours were associated with lower postpartum conception rates. Most importantly, this will save you many hours of sleep!

**Is routine use of oxytocin in the postpartum period justifiable?**

Although twice daily injections of 20 I.U. of oxytocin given daily for ten days postpartum did not alter the rate of uterine involution when compared to untreated mares (as measured by ultrasound measurements). Some investigators suggest that routine ecologic treatment with oxytocin treatment may be beneficial. More data are needed before a general statement can be made on this subject.

**The use of oxytocin at breeding**

In mares that show fluid on ultrasound during estrus and after insemination, the uterus is sometimes flushed with physiological saline, 4 and 8 hours after breeding. Ten to 20 IU of oxytocin is administered immediately after flushing. In the absence of flushing, oxytocin is also given 4 and 8 hours after breeding. If these treatments are delayed until at least four hours after insemination, viable sperm are not removed from the uterus. The stud manager repeats oxytocin treatment at 12-hour intervals after the initial treatment until ovulation is confirmed. Alternatively, mares can be treated IM with a mix of prostaglandin F2alpha, also given at 12 hour intervals after breeding. The rationale for this approach is that oxytocin induces strong uterine contractions for less than 50 minutes but clears intrauterine fluid rapidly. By contrast, the prostaglandin F2 alpha treatment produces low amplitude contractions that persist for up to 5 hours. As reviewed elsewhere, prolonged uterine contractions may assist in lymphatic flow and drainage of uterine fluid. Cloprostenol can be used instead of prostaglandin F2a for this purpose but in either case, treatment should stop before the CL is more than 2 days old, otherwise luteolysis will ensue.
Sperm transport in the female genital tract:
Passage of sperm through the female reproductive tract is regulated to maximize the chance of fertilization and ensure that sperm with normal morphology and vigorous motility will be the ones to succeed. The site of semen deposition is not easy to establish in many species because it must be determined by examining the female immediately after coitus and by considering the anatomy of the penis, vagina and cervix during coitus. However, it has been accomplished for humans, in which semen has been observed pooled in the anterior vagina near the cervical os shortly after coitus. Within minutes of vaginal deposition, human sperm begin to leave the seminal pool and swim into the cervical canal. In contrast, rodent sperm deposited in the vagina are swept completely through the cervix into the uterus along with seminal plasma within a few minutes. Some species, such as pigs, bypass the vagina altogether and deposit semen directly into the uterine cavity, where sperm may quickly gain access to the oviduct. Whereas most of the semen of murine rodents is rapidly transported into the uterine cavity, some remains in the vagina where it coagulates to form a copulatory plug. The plug forms a cervical cap that promotes sperm transport into the uterus. The plugs formed by semen of guinea pigs and mice extend into the cervical canals and thus could form a seal against retrograde sperm loss. Human semen coagulates, but it forms a loose gel rather than the compact fibrous plug seen in rodents. The coagulate forms within about a minute of coitus and then is enzymatically degraded in ½ to 1 h. The predominant structural proteins of the gel are the 50 kDa semenogelin I and the 63 kDa semenogelin II, as well as a glycosylated form of semenogelin II, all of which are secreted primarily by the seminal vesicles. The gel is degraded by prostate-specific antigen (PSA), a serine protease secreted by the prostate gland. It has been proposed that this coagulum serves to hold the sperm at the cervical os and that it protects sperm against the harsh environment of the vagina. Seminal gels are not fully successful at holding sperm at the cervical os. In cattle, several studies have demonstrated loss of sperm from the vagina after mating or insemination, less than 1% of sperm might be retained in the female reproductive tract and this supports the notion that only a minority of sperm actually enter cervical mucus and ascend higher into the female reproductive tract. Like humans, some primates produce semen that forms a soft gel. However, in chimpanzees, a species in which females mate with more than one male in a brief time, the semen coagulates into a compact plug resembling that of rodents. The plug may serve to prevent other males from mating with the female. Some carnivores (e.g. domestic dogs, Canis familiaris) and some rat and mouse species of the family Cricetidae use the penis as a copulatory plug; i.e. the mating pair remains joined together for a period after coitus.

Vaginal defenses against infectious organisms may affect sperm:
The vagina is open to the exterior and thus to infection, especially at the time of coitus; therefore, it is well equipped with antimicrobial defenses. These defenses
include acidic pH and immunological responses and can damage sperm as well as infectious organisms. To enable fertilization to take place, both the female and the male have adopted mechanisms for protecting sperm. In humans, semen is deposited at the external os of the cervix so that sperm can quickly move out of the vagina. Human sperm must contend, however briefly, with the acidic pH of vaginal fluid. The vaginal pH of women is normally five or lower, which is microbicidal for many sexually transmitted disease pathogens. Evidence indicates that the acidity is maintained through lactic acid production by anaerobic lactobacilli that feed on glycogen present in shed vaginal epithelial cells. Lowering pH with lactic acid has been demonstrated to immobilize bull sperm. The pH of seminal plasma ranges from 6.7 to 7.4 in common domestic species and has the potential to neutralize vaginal acid. Vaginal pH was measured by radio-telemetry in a fertile human couple during coitus. The pH rose from 4.3 to 7.2 within 8 s of the arrival of semen; whereas, no change was detected when the husband used a condom. Vaginal washings of women with high levels of detectable seminal antigens had a median pH of 6.1, whereas the median pH of washings lacking detectable antigens was 3.7. Contraceptive gel designed to maintain a low vaginal pH after coitus has been shown to immobilize human sperm in vitro and in vivo. In addition to pH buffers, seminal plasma contains inhibitors of immune responses, including protective components that coat sperm. These are most effective when sperm are bathing in seminal plasma and may be gradually shed when sperm leave the seminal plasma behind. Males may also overcome female defenses by inseminating many sperm. This strategy is particularly effective for overcoming cellular immune responses. In the rabbit, deposition of semen results in an invasion of neutrophils into the vagina. This invasion takes time, however, to build to an effective level. Numerous leukocytes, many containing ingested sperm, were recovered from vaginas of rabbits 3–24 h post coitus. By that time, however, thousands of sperm had already reached the Fallopian tubes.

**Sperm transport through the cervix:**

In some species, the cervical canal widens under the influence of estrogen. Sperm of humans and cattle enter the cervical canal rapidly where they encounter cervical mucus. The extent of hydration is correlated with penetrability to sperm. Coitus on the day of maximal mucus hydration in women is more closely correlated with incidence of pregnancy than coitus timed with respect to ovulation detected using basal body temperature. Cervical mucus presents a greater barrier to abnormal sperm that cannot
swim properly or that present a poor hydrodynamic profile than it does to morphologically normal, vigorously motile sperm and is thus thought as one means of sperm selection. The greatest barrier to sperm penetration of cervical mucus is at its border, because here the mucus microarchitecture is more compact. Components of seminal plasma may assist sperm in penetrating the mucus border. More human sperm were found to enter cervical mucus in vitro when an inseminate was diluted 1:1 with whole seminal plasma than when it was diluted with Tyrode’s medium, even though the sperm swam faster in the medium. Like the vagina, the cervix can mount immune responses. In rabbits and humans, vaginal inflammation stimulates the migration of leukocytes, particularly neutrophils and macrophages, into the cervix as well as into the vagina. Neutrophils migrate readily through midcycle human cervical mucus. In rabbits, neutrophils were found to heavily infiltrate cervixes within a ½ h of mating or artificial insemination. Interestingly, it was discovered that if female rabbits were mated to a second male during the neutrophilic infiltration induced by the earlier mating, sperm from the second male were still able to fertilize. Thus, although the cervix is capable of mounting a leukocytic response, and neutrophils may migrate into cervical mucus, the leukocytes may not present a significant barrier to sperm. It has been demonstrated that neutrophils will bind to human sperm and ingest them only if serum that contains both serological complement and complement-fixing anti-sperm antibodies is present. This can happen in vivo if the female somehow becomes immunized against sperm antigens. Altogether, the evidence indicates that leukocytic invasion serves to protect against microbes that accompany sperm and does not normally present a barrier to normal motile sperm, at least not shortly after coitus. Immunoglobulins, IgG and IgA, have been detected in human cervical mucus. Secretory IgA is produced locally by plasma cells in subepithelial connective tissue. The amount secreted increases in the follicular phase but then decreases just about the time of ovulation. The immunoglobulins provide greater protection from microbes at the time when the cervical mucus is highly hydrated and offers the least resistance to penetration. However, when there are antibodies present that recognize antigens on the surface of ejaculated sperm, infertility can result. Complement proteins are also present in cervical mucus, along with regulators of complement activity. Thus, there is a potential for antibody-mediated destruction of sperm in the cervical mucus as well as leukocytic capture of sperm. Some anti-sperm antibodies are not complement-activating; however, they can still interfere with movement of sperm through cervical mucus by physical obstruction.

Are sperm stored in the cervix?

Vigorously motile sperm have been recovered from the human cervix up to 5 days after insemination, and the presence of sperm in midcycle cervical mucus forms the basis of the ‘post coital test’ (PCT). Nevertheless, it is not known whether sperm collected from cervixes this long after coitus would reach the Fallopian tube and succeed in fertilizing, nor could it be known whether these sperm had re-entered the cervix from the uterus. Very few sperm have been recovered from human uteri 24 h
after coitus. Unless sperm are protected from phagocytosis (and they reservoir to the oviduct 24 h post coitus.

**Sperm transport through the uterus:**
At only a few centimeters in length, the human uterine cavity is relatively small and could be traversed in less than 10 min by sperm swimming at about 5 mm/min, which is the swimming speed of sperm in aqueous medium. Transport of sperm through the uterus is likely aided by proovarian contractions of the myometrium. waves of uterine smooth muscle contractions that increase in intensity during the late follicular phase. Myometrial contractions may be stimulated by seminal components. When vasectomized male rats were mated with females, the incidence of strong uterine contractions declined, indicating that sperm or testicular or epididymal secretions have stimulatory activity. Removal of the seminal vesicles significantly reduced the pregnancy rate in mice. In boars, there is evidence that estrogens, which may reach 11.5 µg in an ejaculate, increase myometrial contraction frequency. Since boar semen is deposited directly into the uterine cavity, the uterus is exposed to the full amount of estrogens in the semen. There is evidence that the estrogens enhance contraction by stimulating secretion of PGF-2α. Rapid transport of sperm through the uterus by myometrial contractions can enhance sperm survival by propelling them past the immunological defenses of the female. As is the case in the vagina and cervix, coitus induces a leukocytic infiltration of the uterine cavity, which reaches a peak several hours after mating in mice. The leukocytes are primarily neutrophils and have been observed phagocytizing uterine sperm in mice, rats and rabbits. This phagocytosis was observed several hours after insemination and therefore might be directed primarily against damaged sperm. However, normal sperm may also be attacked, particularly in vaginal inseminators like humans, because their sperm have lost much of the immune protection afforded by seminal plasma constituents. When sperm first enter the uterus, they outnumber the leukocytes. As time passes, the leukocytes begin to outnumber the sperm. Also, as sperm lose protective seminal plasma coating, they may become more susceptible to leukocytic attack. At some point, even undamaged sperm may fall victim to the leukocytes. Probably, to ensure fertilization, sperm should pass through the uterine cavity before significant numbers of leukocytes arrive.

**Transport through the utero-tubal junction:**
The uterotubal junction presents anatomical, physiological and/or mucous barriers to sperm passage in most mammals. Anatomically, the lumen in species as distantly related as dairy cattle and mice is particularly tortuous and narrow. Within the lumen of the junction, there are large and small folds in the mucosa. In the cow, mucosal folds form cul-de-sacs with openings that face back towards the uterus. This arrangement of folds seems designed to entrap sperm and prevent further ascent. A vascular plexus in the lamina propria/submucosal layer of the wall may create a physiological valve. When engorged, the plexus can compress the lumen. This plexus has been well described in cattle. The walls of the bovine junction and adjacent tubal isthmus also contain a thick muscular layer that could further constrict the lumen. The bovine utero-tubal junction is sigmoidal in shape and supported by muscular ligaments that appear capable of increasing the flexure of the curve and thus compressing the lumen. The narrow lumen of the uterotubal junction may be filled with viscous mucus that can impede the progress of sperm. Mucus has been found in the uterotubal junction in humans, as well as in rabbits, pigs, cattle.

**Rapid sperm transport:**
Sperm have been recovered in the cranial reaches of the tubal ampulla only minutes after mating or insemination in humans and several other species of mammals. Rapid
transport of sperm into the Fallopian tube would seem to counter the proposed model of sperm swimming one-by-one through the utero-tubal junction. However, when rabbit sperm recovered from the cranial ampulla shortly after mating were evaluated, they found that most were immotile and damaged. They proposed that waves of contractions stimulated by insemination transport some sperm rapidly to the site of fertilization, but these sperm are mortally damaged by the associated sheer stress and do not fertilize. Later, motile sperm gradually pass through the uterotubal junction to establish a tubal population capable of fertilizing. The contractions may serve primarily to draw sperm into the cervix but result in overshooting of some sperm. As described above, motile human sperm have been recovered from Fallopian tubes within an hour of insemination; however, it is not known whether function was normal in these women.

**A sperm reservoir in the Fallopian tube:**
As sperm pass through the uterotubal junction and enter the tubal isthmus, they may be trapped and held in a reservoir. The Fallopian tube provides a haven for sperm. Unlike the vagina, cervix and uterus, the tube does not respond to insemination with an influx of leukocytes. In addition to providing a haven, the storage reservoir maintains the fertility of sperm until ovulation. *In vitro*, sperm fertility and motility are maintained longer when sperm are incubated with endosalpingeal epithelium. Entrapment and storage of sperm in the initial segment of the tube may serve to prevent polyspermic fertilization by allowing only a few sperm at a time to reach the oocyte in the ampulla. Sperm numbers have been artificially increased at the site of fertilization in the pig by surgical insemination directly into the ampullar lumen. by resecting the isthmus to bypass the reservoir or by administering progesterone into the muscularis to inhibit smooth muscle contraction of the lumen. In each of these cases, the incidence of polyspermy increased.

**Preserving sperm fertility during storage:**
Sperm–endosalpingeal contact somehow preserves sperm during storage. Human sperm incubated with epithelium *in vitro* remain viable longer than when they are incubated in medium alone, as do sperm from other mammals. Viability of human sperm and other species can be extended by incubating them with vesicles prepared from the apical membranes of the endosalpinx, indicating that the epithelium can produce the effect by direct contact rather than by secretions. It was reported that equine sperm binding to epithelium or membrane vesicles maintain low levels of cytoplasmic Ca2+ compared to free-swimming sperm or sperm incubated with vesicles made from kidney membranes. Human and equine sperm incubated with endosalpingeal membrane vesicles capacitate more slowly than sperm incubated in capacitating medium alone. Possibly, viability is maintained by preventing capacitation and its concomitant rise in cytoplasmic Ca2+. The mechanism for preventing rises of cytoplasmic Ca2+ in sperm are not known, but one suggestion is that catalase, which has been detected in the bovine tube, serves to protect against peroxidative damage to the sperm membranes, perhaps preventing inward leakage of Ca2+. The endosalpingeal binding protein on bull sperm, PDC-109, probably acts to stabilize sperm membranes. PDC-109 reduces membrane fluidity and immobilizes cholesterol in phospholipids membranes, including those of epididymal sperm. PDC-109 can also contribute to membrane stability by inhibiting the activity of phospholipase A2. Thus, PDC-109 may play a role in preserving bull sperm fertility while they are stored in the reservoir.

**Hyperactivation of sperm and the final stages of transport:**
At some point in the female tract, most likely in the Fallopian tubes, sperm become hyperactivated. In aqueous media in vitro, hyperactivated sperm swim vigorously but in circular or erratic patterns. In vivo, the physical environment encountered by sperm is quite different and evidence indicates that hyperactivation is required by sperm to progress towards the oocyte and penetrate its vestments. As discussed above, hyperactivation may assist sperm in detaching from the endosalpingeal epithelium. In addition, hyperactivation enhances the ability of sperm to swim through viscoelastic substances such as mucus in the tubal lumen and the extracellular matrix of the cumulus oophorus. Mucus fills the utero-tubal junction and extends into the isthmus in humans, rabbits, pigs and dairy cattle. Hyperactivated sperm penetrate artificial mucus, such as viscoelastic solutions of long-chain polyacrylamide or methylcellulose, far more effectively than non-hyperactivated sperm.

**Taxis of sperm towards oocytes:**
Although the existence of a guidance system to help mammalian sperm reach the unfertilized oocyte has been debated over the years, stronger evidence for such a system has surfaced recently. There is evidence for the existence of two complementary guidance mechanisms operating within the Fallopian tube. The first (long-range) mechanism is where capacitated sperm—released from intimate contact with the endosalpinx are guided by thermotaxis towards the site of fertilization. A temperature difference of up to 2°C between the cooler tubal isthmus and the warmer tubal ampulla has been detected in rabbits and there are indications that capacitated rabbit sperm tend to swim towards warmer temperatures. Once in the tubal ampulla, and at a closer proximity to the oocyte, a second (short-range) chemotactic mechanism may guide sperm closer to the oocyte. Sperm are equipped with a mechanism for turning towards the oocyte in response to chemotactic factors; that is, they can switch back and forth between symmetrical flagellar beating and the asymmetrical flagellar beating of hyperactivation. Hyperactivation is reversible, so sperm can alternate between turning and swimming straight ahead. Mammalian sperm have been reported to turn towards, or accumulate in, a gradient of follicular fluid, which could accompany the oocyte into the Fallopian tube. Nevertheless, the chemotactic agent in follicular fluid has not been identified, nor has its presence in the Fallopian tube been detected. Odorant receptors unique to sperm have been localized to a spot on the base of the flagellum of human, canine and rat sperm. Placing human sperm in a gradient of the odorant scented oil caused them to orient into the gradient and triggered a calcium and cAMP-mediated signalling cascade. Nevertheless, a chemotactic odorant has yet to be identified in humans or other mammals. If one were found, it could have vast implications for the development of contraceptives, as well as assessment and treatment of infertility.

**The fate of non-fertilizing sperm:**
After fertilization, any sperm remaining in the female reproductive tract may be phagocytosed by isthmic epithelial cells or may be eliminated into the peritoneal cavity where they are phagocytosed. Phagocytosis within the Fallopian tubes may be primarily employed by species, such as mice, which have an extensive ovarian bursa that would limit passage of sperm into the peritoneal cavity. In species where the passage of sperm into the peritoneal cavity is possible, this does not quickly render sperm non-functional as evidenced by the numerous case reports of human tubal pregnancies that arose in spite of lack of access of sperm from the uterus into the oviduct on the side of ovulation. In these cases, the only route available to the sperm was through the peritoneal cavity.

**Fertilization and conception:**
At ovulation the ovum or egg is collected by the fundibular end of the oviduct or fallopian tube. It is transported down the oviduct towards the uterus possibly by a combination of cilial (hair-like) action and muscular contractions. Transport through the oviduct appears to be under the control of ovarian steroid hormones since oestrogens reduce and progesterone increases the speed of passage of ova through the oviducts. Fertilization normally occurs in the ampulla section of the oviduct close to the junction with the isthmus. In the cow, the ovum enters the uterus 4–5 days after ovulation. Mammalian spermatozoa acquire motility, and part of their capacity to fertilize the ovum, during their passage through the epididymis. At the same time, they undergo changes in metabolic patterns, enzymatic activities, the ability to bind to zona pellucida surface, electrophoretic properties, and stabilization of some sperm structures. However, before spermatozoa are able to fertilize the ovum, they have to undergo a further series of maturational changes in the female tract. These processes are known as capacitation and the acrosome reaction and are thought to require about six hours in the cow. This requirement for maturational changes is the main reason why it is preferable to inseminate cows several hours before ovulation. The precise changes involved in capacitation are not fully understood, but they involve enzymic and structural modifications to the acrosome and anterior part of the sperm head membrane. These include:

1. an increase in membrane permeability to calcium
2. modification of the membrane structure
3. activation of the enzyme adenyl cyclase
4. conversion of the protein proacrosin to acrosin.

The process of capacitation is stimulated when sperm enter the female reproductive tract. The acrosome reaction follows capacitation and involves the fusion of the sperm cell membrane and the acrosome and the formation of gaps through which the acrosome contents can diffuse. The acrosome reaction is necessary to allow penetration of the oocyte by the sperm. Capacitation and the acrosome reaction are very closely linked and therefore it is not always possible to distinguish between the two processes. The presence of ovarian follicular fluid and the cumulus oophorus have a stimulatory effect on the acrosome reaction but do not appear to be essential for it. On reaching the ovum, the sperm penetrates any remaining cumulus oophorus by the action of the enzyme hyaluronidase from the acrosome and comes into contact with the zona pellucida. The sperm nucleus possesses a cytoskeletal coat, the perinuclear theca (PT), which is removed from the sperm head at fertilization. The PT contains an oocyte-activating factor. This has not been characterized, but is thought to be responsible for triggering the signaling cascade of oocyte activation (Sutovsky, 2003). Mobility of the spermatozoa is also important in the process of sperm penetration. Normally, only one sperm is able to pass through the zona, but when more enter, a process known as polyspermy, the resultant embryo is non-viable. Following fusion of sperm and egg, the contents of the cortical granules in the egg release into the perivitelline space (the cortical reaction),
causing the zona pellucida to become refractory to sperm binding and penetration (the zona reaction). Several ways in which the cortical reaction may be mediated. It was demonstrated that the ability of the zona pellucida to prevent the entry of another spermatozoon after fertilization persisted through to the blastocyst stage. The fusion of the sperm and ovum cell membranes begins at the middle of the sperm head region. The sperm head becomes engulfed by the ova with the loss of the tail. The sperm’s nuclear membrane disappears and the male chromatin comes into contact with the ova cytoplasm. Penetration by the fertilizing sperm (pronucleus) stimulates the resumption of the second meiotic division of the oocyte and the extrusion of the second polar body. Fertilization is completed with the fusion of the haploid male and female pronuclei, a process known as syngamy.

**PHASES OF FERTILIZATION:**

1. Passage of sperm through the corona radiata of the oocytes by the use of hyaluronidase which is released by the acrosome of the sperm and movement of the tail.
2. Penetration of Zona Pellucida helped by the enzymes esterase, acrosin, and neuraminidase. Once penetration occurs, a biochemical zona reaction causes the egg to become impermeable to other sperm.
3. Fusion of the plasma membranes of the oocyte and sperm.
4. Completion of second meiotic division of the oocyte and formation of the female pronucleus.
5. Formation of the male pronucleus
6. Breakdown of pronuclear membranes, condensation of chromosomes, and arrangement of chromosomes for mitotic cell division.

**Folliculogenesis and ovulation:**

The process of follicular development occurs continuously throughout the estrous cycle. The follicles are developed in waves throughout the estrous cycle. There are two types of follicular waves: an anovulatory wave and ovulatory wave. The only difference between these two or three waves (one to two anovulatory waves and one ovulatory wave) is the timing of the wave in the estrous cycle. In the ovulatory wave the levels of LH continue to increase and result in an LH surge, causing the ovulation of the oocyte. Folliculogenesis is the development of the follicles and then the recruitment, selection, dominance/ovulation, and atresia of an antral follicle(s) in the ovary. The number of follicles that develop and ovulate somewhat depends on the species. Some species are polytocous (litter-bearing; multiple ovulation), whereas others are monotocous (one offspring; single ovulation). There are different types of ovarian follicles that represent the different stages of development and maturity at any time of the estrous cycle. There are four stages of follicles present in the ovary: primordial follicle, primary follicle, secondary follicle, and antral follicle. Follicle growth and development result from a complex interaction between the hypothalamic-pituitary axis and the ovaries. In addition, local factors in the ovary influence this process through paracrine/autocrine regulation. From the hypothalamus, Gonadotrophin Releasing Hormone (GnRH) is transported to the anterior pituitary where it stimulates the production and the release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). These latter two hormones stimulate follicular growth in the ovary. Generally, the bovine estrous cycle exhibits two or three waves of follicular growth. Each new follicle wave is preceded by an FSH surge since follicles require FSH support to grow beyond a size of 4 mm diameter. In a follicular wave, each follicle has the capacity to acquire dominancy, but the future dominant follicle starts to grow 6 h before the future subordinate ones and, therefore, has a
small size advantage. Selection of the dominant follicle and deviation occur when the largest follicle reaches a size of \( \approx 8.5 \) mm. At deviation, the largest follicle (dominant) continues to grow while the growth rate of the smaller follicles (subordinates) decreases. At this time the FSH-concentration has fallen below a critical level necessary to sustain follicle growth. However, the dominant follicle acquires even more LH receptors in the granulosa cells, enabling the use of increased LH-concentrations for continued growth at the time of selection. Also, the dominant follicle further reduces FSH release through a negative feedback by oestradiol and inhibin, thereby preventing the continued growth and development of any subordinate follicles. In addition, the future dominant follicle exhibits changes in the Insulin-like Growth Factor (IGF)-System before the processes of selection and deviation. Decreased concentrations of binding proteins increase the bio-available fraction of IGF-1 and 2 in the follicular fluid of the, dominant follicle. Both growth factors stimulate cell proliferation and oestradiol production and augment granulosa cell sensitivity for FSH, thereby enabling the dominant follicle to further use the now very low FSH concentration and to continue growth. Other intrafollicular factors such as inhibin, activin and follistatin may or may not play a role in the processes of selection and deviation of the dominant follicle. The dominant follicle will further grow and mature and will either ovulate, become atretic or develop into a cyst. To be able to ovulate, the dominant follicle must elicit a pre-ovulatory LH-surge through increased oestradiol production. Pulsatile secretion of luteinizing hormone (LH) from the pars distalis region of the anterior pituitary gland induces an increase in prostaglandins and various collagenases that enzymatically degrade the follicular wall. Thus, the LH pulse culminates in ovulation of the tertiary follicle. The cumulus oophoros, corona radiata, and primary oocyte are released from the follicle into the ovarian bursa and peritoneal cavity, and finally transcend into the ampulla of the oviduct to await fertilization. During this time, the primary oocyte completes the first round of meiosis. A corpora hemorrhagica (characterized by hemorrhage into this area) forms after the follicular wall collapses. The remaining granulosa and theca cells undergo luteinization and fill in this open area to form a corpora lutea. Cats, llamas and other species are induced ovulators and thus, the act of copulation is necessary to trigger ovulation. Most other domestic animal species ovulate spontaneously. The second meiotic division is completed at the time of fertilization. Progesterone inhibits LH secretion, and thus high concentrations of this steroid hormone inhibit ovulation.
Cystic Ovary:
Cystic ovarian follicles (COF) are an important cause of subfertility in dairy cattle, as they extend the calving interval. Prolongation of the calving interval and treatment costs of COF result in economic loss for the dairy farmer. In most of the literature, COF are referred to as Cystic Ovarian Disease (COD). However, this terminology should be revised since the emphasis on cystic follicles has shifted over time. In the 1940’s, the presence of cystic follicles on the ovaries was mainly associated with nymphomania and a bull-like appearance in cows, which are clear clinical signs of a state of “disease”. Over the past decades, dairy herd management and economics have evolved to a situation in which normal functioning of the ovaries in the postpartum period is utterly important. During this period, cystic follicles are rather common, and generally occur without obvious clinical signs. Normal ovarian cyclicity is however delayed and these cysts should therefore be regarded as COD, despite the absence of signs of disease in the majority of cases. In addition, after a variable period of time cysts can become non-steroidogenic and then they no longer interfere with cyclicity. Consequently, at the time the non-steroidogenic cyst is observed, no other clinical abnormalities are present. Conclusively, in the present-day dairy herd health programs “cysts” are often diagnosed in the absence of clear clinical signs. Therefore the term “Cystic Ovarian Disease” does no longer seem appropriate and should be replaced by the term “Cystic Ovarian Follicles” which does not necessarily implicate a state of disease. In this review, we will therefore use COF instead of COD. We prefer to use COF instead of “ovarian cysts”, because the former term indicates that it is the ovarian follicle(s) and not any other ovarian tissue that becomes cystic.

Definition: Cystic ovarian follicles develop when one or more follicles fail to ovulate and subsequently do not regress but maintain their growth and steroidogenesis. They are defined as follicle-like structures, present on one or both ovaries, with a diameter of at least 2.5 cm during a minimum of ten days in the absence of luteal tissue. It has become clear though that this definition needs to be revised. First, the size limit is rather artificial as follicles might already become cystic at a smaller size, and dominant follicles ovulate on average at a size of 1.6 to 1.9 cm in dairy cows. Moreover, many researchers showed that COF are actually dynamic structures, which can regress and be replaced by new cysts. So the required individual persistency of ten days is questionable. In addition, in practice veterinarians generally do not have the opportunity to perform a second examination of an animal ten days after the initial diagnosis of COF to fulfill all the terms of the definition. The absence of a corpus luteum is another requirement, which is not always fulfilled. Non-steroidogenic cysts which are hormonally inactive do not influence the normal estrous cycle, so they can occur together with a corpus luteum. Therefore, recent research articles define COF differently and perhaps more logically, although a generally accepted definition is still lacking, which can also be attributed to the heterogeneity (type of cyst, time of occurrence, clinical signs) of the cysts. Based on the current knowledge and recent literature, COF may be defined as follicles with a diameter of at least 2 cm that are
present on one or both ovaries in the absence of any active luteal tissue and that clearly interfere with normal ovarian cyclicity. Macroscopically, cysts can be subdivided into follicular and luteal cysts, which are considered to be different forms of the same disorder. The former probably evolve into the latter and consequently many intermediate forms exist with limited or extensive luteinization of the follicle wall. Determination of progesterone concentrations in blood plasma, milk or milk fat can help to make a distinction between the two types. Follicular cysts secrete little or no progesterone while luteal cysts clearly do. However, the threshold values used in literature differ a lot, which makes it difficult to set a concentration threshold. And as mentioned, the many intermediate forms do not allow for a clear identification of cyst type. So classification is not easy and is subject to personal interpretation. Ultrasound can be useful in supplying extra information. Follicular cysts have a thin wall (≤ 3mm) and the follicular fluid is uniformly anechogenic, while luteal cysts have a thicker wall (> 3mm), which is visible as an echogenic rim. Also, the latter often have echogenic spots and web-like structures in the follicular fluid. Follicular cysts initially continue to produce estrogen in the absence of other follicles >5 mm on ultrasound. After a variable period of time estrogen production may cease. The cyst becomes non-steroidogenic without luteinizing, thereby allowing a new follicular wave to emerge and follicles to grow beyond 5 mm.

**Incidence and Symptoms:**
Cystic ovarian follicles can occur at different times throughout lactation. The incidence varies between 6 and 30%. The diagnosis of COF is most often made during the first 60 days post partum, mainly because of the close monitoring of cow fertility during this period. The majority of all cysts occur throughout this stage. The self-recovery percentage of these early cysts is 60-65%. Despite this high self-recovery rate, the importance of dairy cow fertility is not negligible. By delaying normal cyclicity, the time to first insemination increases and pregnancy rates after first insemination decrease. A genetic predisposition exists for COF but the heritability is rather low, being 0.07 to 0.12. However, the incidence in Dutch Holstein Friesian herds is actually increasing. Genetic selection to reduce the incidence of COF can be successful, despite the low heritability. By excluding bulls, which sired daughters with cysts, from breeding programs, cyst incidence was reduced by 50% over a 20-year period in a Swedish cattle population. The clinical signs that accompany ovarian cysts are variable. Anoestrus often occurs, especially during the postpartum period and both with follicular and luteal cysts. Irregular estrus-intervals, nymphomania, relaxation of the broad pelvic ligaments and development of masculine physical traits are other signs, which may be present, especially brief during lactation. According to recent literatures, 80 % of all cows suffering from COF are anoestrous while the remaining 20% are nymphomaniac, but this division seems too strict since many different signs may accompany cysts.

**Pathogenesis of ovarian cysts:**
Ovarian dysfunctions like cysts occur most often during the early postpartum period when there is a transition from the non-cyclic condition during pregnancy to the establishment of regular cyclicity. It is generally accepted that cystic follicles develop due to a dysfunction of the hypothalamic-pituitary-ovarian axis. This dysfunction has a multifactorial etiology, in which genetic, phenotypic and environmental factors are involved. When discussing the pathogenesis of COF, a distinction may be made between a primary defect in the hypothalamus-pituitary and a primary defect at the level of the ovary in the follicle itself.

**Hypothalamic-pituitary dysfunction**
The most widely accepted hypothesis explaining the formation of a cyst is that LH release from the hypothalamus-pituitary is altered: the pre-ovulatory LH-surge is either absent, insufficient in magnitude or occurs at the wrong time during dominant follicle maturation, which leads to cyst formation. This unusual LH release does not seem to be caused by a lower GnRH content of the hypothalamus, nor by reduced GnRH receptor numbers or LH content in the pituitary. It is believed that an altered feedback mechanism of oestrogens on the hypothalamus pituitary can result in an abnormal GnRH and LH release and cyst formation. A GnRH/LH surge prematurely occurring during follicle growth, i.e. when no follicle capable of ovulation is present, can render the hypothalamus unresponsive to the feedback effect of oestradiol which results in the formation of ovarian cysts. To restore the feedback mechanism, the hypothalamus needs to be exposed to progesterone. Very recently, it was shown that a similar state of hypothalamic refractoriness to oestrogens and subsequent cyst formation can be achieved if the increase in progesterone after a spontaneous ovulation is prevented. This physiological state of hypothalamic unresponsiveness to oestrogens seems to be present in the majority of cows with COF, as illustrated by the failure of an exogenous oestradiol treatment to elicit a timely LH surge. On the other hand, the refractoriness of the hypothalamus-pituitary for oestradiol in cows with COF seems to be a consequence rather than a cause of the disease. Removal of the cystic ovary by ovariectomy restores the feedback mechanism and the capacity of oestradiol to elicit an LH-surge, although the underlying mechanism is not know. An altered feedback mechanism and GnRH/LH release may be attributed to factors interfering at the hypothalamic-pituitary level. Progesterone at suprabasal concentrations blocks the LH-surge, thereby inhibiting ovulation but increasing the LH pulse frequency. This results in an anovulatory, persistent follicle with a larger diameter and a longer lifespan than normal, and increased peripheral oestradiol concentrations. These follicular and hormonal changes are very similar to observations made in cows with COF. Recently, found that at the time of diagnosis, most cysts are accompanied by suprabasal progesterone concentrations, which play a role in cyst turnover. These observations together with the similarities between persistent follicles, induced by suprabasal progesterone, and naturally occurring cysts, suggest a role for progesterone in the pathogenesis of COF. Factors indirectly reducing GnRH/LH secretion like stress, intraréine infections and seasonality are also considered to increase the risk of cyst formation. In cystic cows, increased LH pulse frequencies and amplitudes accompany the formation of new cysts. However, hypersecretion of LH does not seem to be involved in cyst formation, but it may play a role in cyst persistence. Data obtained in sheep also dismiss an increased LH secretion as a primary cause of COF. In conclusion, an aberrant LH-surge is likely the trigger for the development of COF. The abnormal LH release seems to be caused by an altered feedback mechanism of oestrogens on the hypothalamus-pituitary. The malfunctioning of the feedback mechanism can be caused by factors directly interfering at the hypothalamic-pituitary level or by an altered follicle growth and development disrupting the hypothalamic-pituitary-gonadal axis, as discussed below.

**Ovarian/Follicular dysfunction**

A primary dysfunction at the level of the follicle may disrupt the hypothalamic-pituitary ovarian axis and cause the formation of COF. First of all, alterations in LH receptor expression and content may cause anovulation of the follicle. The LH-surge initiates a complex multi-gene, multi-step process in which timing is essential, finally leading to ovulation of the pre-ovulatory follicle. FSH and LH receptor numbers in granulosa cells of cysts are decreased when compared to normal follicles, but this is
contradicted others they found similar receptor concentrations or higher levels of receptor mRNA expression, respectively. These discrepancies between studies may partly be explained by differences in methodology such as demonstration of the receptor itself or its mRNA, and the division of cysts into estrogen-active and oestrogen-inactive. Still, such studies are incapable of clearly establishing a cause-effect relationship, since any detected changes may be primary or secondary to cyst formation. However, no significant differences in FSH/LH receptor mRNA were observed between these young cysts and dominant follicles, indicating that the increased LH mRNA expression in oestrogen-active cysts is a consequence rather than a cause of the cystic state. Young cysts were, however, studied in the presence of existing cysts, i.e. when the endocrine environment was already altered, and therefore the pathogenesis may differ from primary developing cysts. Another receptor of interest is the estradiol receptor β (ER-β). In rodents, the importance of this receptor in follicular growth and development has clearly been demonstrated and its localization in follicle cells throughout follicular development has been described in many mammals including cattle. More specifically, in rat ovariol follicles ER-β mRNA expression precedes increased expressions of mRNAs for the LH receptor and specific steroidogenic enzymes. Therefore, alterations in expression of the ER-β might disrupt the local intra-ovarian paracrine/autocrine system, leading to an altered follicular development and steroidogenesis and finally formation of COF. However, his hypothesis is not supported by some data showing that ER-β mRNA expression was not altered in growing cysts. Decreased oestrogen receptor concentrations in follicular cysts, but the oestrogen receptor type was not defined, and once again this may be either cause or effect of the disorders. Besides changes in receptor expression and content, alterations in steroidogenesis by the dominant follicle may also play a role in its cystic degeneration. After all, the dominant follicle has to elicit an LH surge at the right time in its development by producing sufficient estradiol. Oestrogen-active cysts show a higher expression of 3β-hydroxysteroid dehydrogenase mRNA, a steroidogenic enzyme, and cows developing a cyst have increased estradiol concentrations during the early stages of follicular dominance. However, no one was able to observe changes in mRNA expression of steroidogenic enzymes in the follicle wall of young growing cysts. It could be concluded that alterations of the endocrine system precede and perhaps cause, the observed follicular alterations in cysts. However, developing in the presence of existing cysts, i.e. when the endocrine environment was already altered. As a consequence, the mechanism causing these "young cysts" to actually become cysts may differ from the mechanism(s) involved in primary cyst formation. Steroidogenesis is enhanced during early development of future cysts, which may disrupt the hypothalamic-pituitary-gonadal axis. Through an increased positive feedback, LH release would be over stimulated and the final LH-surge would occur too early during follicle development. Due to immaturity, the follicle would not be able to respond with ovulation and may then become cystic. Apart from changes in mRNA expression for certain receptors and steroidogenic enzymes, cell proliferation and apoptosis in the granulosa and theca interna cell layers also seem to be altered in cystic follicles. Early cystic follicles show an increase in apoptosis while cell proliferation is decreased. Although it is hard to establish a cause-effect relationship, alterations like these may disrupt normal follicle growth and steroidogenesis leading to cystic degeneration. Others suggested that matrixmetalloproteinases (MMPs) could be involved in the formation of cysts: higher proMMP-2 and -9 levels were present in the follicular fluid of cysts than in the follicular fluid of normal dominant follicles. MMPs play a role in follicle wall
remodeling and rupture at the time of ovulation but hereto the inactive pro-MMP form needs to transform to the active MMP form. This activation is triggered by the LH-surge. Since an aberrant LH-surge causes COF formation, the higher proMMP-2 and -9 levels in the follicular fluid of COF are most likely an indication of the lack of an LH-surge rather than a cause of COF formation. Although studies focusing on differences between cystic and normal dominant follicles have greatly enhanced our knowledge, it is not possible to determine a cause-effect relationship. Therefore, future research should try to elucidate if, and what kind of, changes during follicle growth can interfere with normal follicle development and steroidogenesis finally leading to the formation of (a) cyst(s). However, due to the inability to predict the fate of a follicle (ovulation/atroresia/COF), studying the follicular changes prior to natural cyst formation is almost impossible. Development of an accurate model for cyst induction, which mimics the in vivo situation, is therefore necessary, but extremely difficult. Perhaps an intensive monitoring of the growth and development of follicles, which finally become cystic may reveal specific characteristics that allow for the classification of a follicle as a future cyst before it actually becomes one. This would offer the opportunity to identify follicular changes early on in cyst development.

**Predisposing factors for COF:** COF are mainly observed in high yielding dairy cows during the first months post partum and milk yield is generally considered a risk factor, although not all authors agree. Moreover, besides the fact that COF are hereditary, a genetic correlation between cysts and milk production traits was established, indicating that an ongoing selection for production parameters will increase the incidence of COF. What the genetic factor(s) is and how it promotes the formation of cysts is not known. However, the fact that cows do not develop a cyst during every lactation and during every ovarian cycle indicates that the gene(s) expression may be promoted by, or gains functional importance under, certain stressors, for example high milk yield and the associated negative energy balance (NEB) during the early postpartum period. At this time, energy demands to sustain milk yield are higher than energy intake thus causing a NEB. This NEB is accompanied by several hormonal and metabolic adaptations, affecting ovarian function. Energy balance may be a more accurate parameter than milk yield to further elucidate the association between COF and production traits. Some animals can compensate for higher milk production through greater dry matter intake reducing the effect of milk yield on energy balance. This could explain why not all authors observed a correlation between ovarian cysts and milk yield. However, when focussing on energy balance and the occurrence of COF, results still remain inconclusive. Other researchers observed a deeper NEB and increased mobilization of body reserves in cows developing cysts, the nadir of the NEB occurred later post partum in cystic cows than in ovulatory cows. Moreover, cystic cows even mobilized less body reserves and derived a smaller percentage of their milk yield from body weight loss. They were unable to find a more severe NEB, evaluated by the fat/protein ratio in milk, in cows with COF compared to ovulatory cows. However in an earlier study, Other observed that a high fat/protein ratio, and, therefore, a more severe NEB, increased the risk of cyst occurrence. Data in sheep also suggest that an increased mobilization of body reserves, indicative for a deeper NEB, is linked with the occurrence of cystic follicles. Although a consensus is lacking, we conclude from literature that a link seems to exist between COF and the magnitude and/or duration of the NEB. The possible underlying mechanism(s) is(are) also still unclear, but NEB may affect COF formation at both the level of the hypothalamus/pituitary and the ovary/follicle through associated hormonal and metabolic changes. During NEB,
circulating concentrations of IGF-1, insulin, glucose and leptin are reduced, while concentrations of metabolites such as non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) are increased. As mentioned earlier, the IGF system plays an important role in follicle growth and development. Besides a direct effect, IGF-1 together with insulin indirectly stimulates follicular development through upregulation of the LH-receptor on granulosa cells. Therefore, low systemic IGF-1 concentrations early post partum could contribute to anovulation and subsequent development of cystic follicles. However, no one could confirm this hypothesis. In addition, insulin itself is known to be a potent stimulator of follicle cell steroidogenesis and proliferation in vitro and in vivo. As a result, reduced circulating insulin concentrations early post partum may play a role in ovarian dysfunction i.e. cyst formation. Besides low insulin concentrations, a general state of peripheral insulin resistance is present as well in high yielding dairy cows early post partum. Insulin resistance is regarded as an important factor in the pathogenesis of the Polycystic Ovary Syndrome in women and COF have often been compared to this syndrome, justified or not. However, rather insulin insufficiency not insulin resistance has been observed in COF cows, indicating an altered interaction between glucose and insulin at the pancreatic level. In addition, in ewes it was not possible to induce cyst formation through establishment of a state of insulin resistance. Conclusively, IGF-1 and insulin are important stimulators of follicle growth and based on the limited number of publications on the subject, low concentrations of one or both of the hormones may contribute to the formation of COF. Further research should confirm whether or not this hypothesis is valid. Leptin is a recently “new” hormone, produced by adipocytes, and is regarded as the ultimate factor linking metabolic status to reproduction. Depending on the metabolic state of the animal it has either a stimulatory effect or none at all on hypothalamic-pituitary function in cattle. There is a hypothesis that above a certain threshold level, leptin acts as a trigger to initiate hypothalamic-pituitary gonadotropin secretion. Besides effects on the central nervous system, this hormone also seems capable of modulating ovarian function by acting directly on follicular cells. Both bovine granulosa and theca cells possess leptin receptors and in vitro leptin inhibits insulin stimulated steroidogenesis of granulosa and theca cells. However, both basal, IGF-1 and LH-stimulated steroidogenesis and cell proliferation as well as insulin-stimulated cell
growth are unaffected by leptin. This indicates that in a low leptin environment (i.e. poor body condition and poor nutrition), ovarian function is mainly regulated by gonadotropins and low insulin/IGF-1 concentrations. In a moderate to high leptin environment, as in obesity, leptin will limit ovarian steroidogenesis, stimulated by the high insulin/IGF-1 concentrations, to prevent overproduction. In the postpartum dairy cow, a clear relationship between leptin profiles and first postpartum ovulation is lacking, although a minimum permissive level of leptin seems required to induce the first postpartum LH-surge. Therefore, leptin may play a role in cyst development. Cows developing cysts have higher serum NEFA concentrations during the first week(s) post partum than ovulatory cows. In rats, elevated NEFA concentrations for 48 h can decrease insulin secretion by the β-cells of the pancreatic islets in response to a glucose challenge. Moreover, NEFA are cytotoxic for several cell types, including human granulosa cells. So (prolonged) exposure to high NEFA concentrations during periods of NEB may hamper follicle growth and development through these mechanisms, disrupting the complex endocrine system and promoting the formation of ovarian cysts. Besides NEFA, increased serum ketone concentrations also affect ovarian function, indicating that these metabolites may be mediators of the negative effect of NEB on follicular development. High ketone concentrations increase the risk of cyst occurrence and consequently are likely candidates to be involved in the formation of COF.

**Treatment:**
The earliest method of treating cysts was by manual rupture per rectum. Although rupture sometimes occurs inadvertently, it should not be done intentionally as it can cause trauma or haemorrhage, which might result in ovaro-bursal adhesions. Surgical removal of one chronically affected ovary or puncturesis using a long hypodermic needle through the sacrosciatic ligament might be worth considering in a limited number of cases where other treatments have failed. Most cysts are now treated using reproductive hormones. The choice of hormonal treatment regimen depends largely upon the type of cyst that is present; follicular cysts are usually treated with either gonadotrophic hormones (i.e. hCG or GnRH) or progesterone, whereas luteinised cysts are normally treated with luteolytic substances. The first successful treatment of follicular cysts was with unfractionated sheep pituitary extract. Subsequently, intravenous hCG has been used. The hCG is usually given by the intravenous route, at doses of between 5000–10 000 i.u. to 10 000 i.u. GnRH has also been used successfully to treat follicular cysts. It was thought at first that GnRH or hCG administration caused luteinisation of the cyst either by inducing an increase in endogenous LH secretion or by causing luteinisation directly. However, it is increasingly well recognised that GnRH has little direct effect upon the cyst itself but, instead, it causes ovulation of new follicles. These follicles develop into corpora lutea. Thus, whether GnRH induces luteinisation of the cyst or the formation of new corpora lutea, the result is an increase in progesterone concentrations, usually within 10 days of treatment. Elevated progesterone concentrations cause a negative feedback-induced decline in endogenous LH secretion. A consequential decline in follicular steroid synthesis occurs, leading to declining oestradiol-17β concentrations. This is considered to be the most important factor in restoring normal cyclical activity. Doses of 100–250 µg of GnRH probably cause luteinisation of the cyst. The most logical way to treat a luteal cyst is the use of PGF2α, although there is still no explanation for the failure of cows to respond to their endogenous luteolysin.
Consequences of cystic ovarian disease
Cystic ovarian disease depresses fertility in a number of ways; it extends the calving interval, decreases lifetime milk yields and increases the involuntary culling rate. The development of mucometra, in which there is distension of the uterus with mucoid fluid and thinning of the uterine wall.

Anestrous:
Physiological anoestrus
It is important to bear in mind that during certain phases of the reproductive cycle, the absence of estrus is physiological. These phases are prior to puberty, during pregnancy and for at least two weeks after calving. It is not uncommon for a cow that is pregnant to be presented to the veterinarian as a case of anoestrus. This occurs if there is unknown access to a bull, or unrecorded insemination or misidentification of the cow inseminated. In dairy cows, from which the calves are removed a day or so after birth, ovulation rarely occurs earlier than 15 days after calving; in general, first postpartum ovulation occurs 17–21 days after calving in medium-yielding dairy cows calving in appropriate body condition and experiencing no major loss in body condition subsequently. This postpartum interval to first ovulation may be prolonged to 60 days in high yielding cows. In cows that are suckling, usually beef cows, the duration of noncyclicity may be extended from 25 to beyond 100 days, depending on body condition score at calving and postpartum nutrition. The effect of suckling of one or more calves is to inhibit the return to cyclicity via neurohormonal routes, which can be modified by nutritional factors. This is demonstrated by the observation that some well-fed, well-managed beef suckler herds achieve a tight calving pattern at the same time each year, which requires an early return to cyclicity while the cows are still suckling their calves. For a list of factors that affect the onset of puberty.

- Physiological anoestrus: prepuberty, pregnancy, puerperium
- True anoestrus: no estrous cycle and no ovulation or blocked cycle (prolonged luteal phase, such as with pyometra)
- Silent estrus: better termed silent ovulation, i.e. ovarian cycles occur but no estrous behaviour is detected
- Weak estrus: poor estrous expression (reduced length or intensity)
- Unobserved estrus: estrus normal, human error

The early resumption of estrous cycles following calving is important for high reproductive efficiency in both year-round and seasonally calving herds. For pasture based production systems, timing of calving is usually set to optimize the use of maximum pasture growth rate in spring and early summer. To maintain the required concentrated calving pattern, high submission rates during the early part of the breeding period are an important prerequisite. To maintain a 365-d calving interval, cows need to conceive on average by 83 d after calving (assuming gestation length of 282 d). Delays in the commencement of ovulation and expression of estrus are associated with reduced conception rates, pregnancy rates, and increased intervals from calving to conception. This is a result of fertility following insemination being greater in cows that have displayed estrus once or more before the start of breeding compared with cows inseminated at their first estrus. Moreover, cows that have not been observed in estrus during the first 60 d after calving have a significantly higher risk of being culled than cows that have displayed estrus. In both average and high yielding dairy cows, there is an economic benefit in reducing the numbers of animals with an extended interval from calving to conception. In dairy herds with seasonal breeding systems, cows not detected in estrus by a specific calendar date, which is nominated as the start of the breeding period, are defined as anestrous. These animals
may have delayed ovulation (anovulatory anestrous) or have ovulated without being detected in estrus, or have calved late and not had sufficient time to resume estrous cycles postpartum. Herds with extended calving patterns may have a large proportion of anestrous cows at the start of the breeding period, simply due to a high proportion of late calving cows. In practical terms, all cows not having displayed estrus at the start of the breeding period need to be examined and treated, irrespective of calving date, in order to maintain the seasonal calving pattern. Anovulatory anestrous cows have a lesser percentage of animals detected in estrus in the first 3 wk of the breeding period (55 vs. 96%) and longer intervals to conception (37 vs. 22 d) than cows that have displayed estrus by the start of the breeding period. Between 10 and 30% of cows that have not been detected in estrus by the start of the breeding period have a detectable corpus luteum at veterinary examination? These cows have reduced pregnancy rates in the first 28 d of the breeding period (59 vs. 67%) and have greater non-pregnancy rates at the end of the breeding period (10 vs. 4%) compared with cows that have been detected in estrus.

**THE POSTPARTUM ANESTROUS PERIOD**

Anovulatory follicular waves occur periodically during pregnancy, with the emergence of follicles of up to a maximum of 6 mm in diameter. However, because of the prolonged period of inhibition during pregnancy, due to the continuous negative feedback effect of progesterone secreted by the corpus luteum and placenta, the pituitary is refractory postpartum, as demonstrated by a lack of response to the administration of gonadotropin releasing hormone (GnRH). This eventually recovers with time. As a result of the absence or low output of gonadotrophins the ovary is relatively quiescent and the cow is in the anestrous phase, which may be prolonged in suckler and high-yielding cows. However, during this postpartum phase the ovaries frequently contain numerous large anovulatory follicles which quickly become atretic; these are sometimes incorrectly diagnosed as cysts. In the immediate postpartum period both oestradiol and progesterone are low. The anterior pituitary is capable of releasing FSH during the first few days postpartum so that with the sporadic release of endogenous GnRH there is a gradual and sustained rise in plasma FSH. After about 7–10 days, this is sufficient to result in the emergence of the first follicular wave; this occurs at about 4 days in dairy cattle, and 10 days in beef cattle. The ability of the pituitary to release luteinising hormone (LH) is much slower, for although the early release of GnRH causes some rise in LH, it quickly returns to basal levels. If a very large dose of endogenous GnRH is given within 10 days of calving there is no release of LH; if standard doses of GnRH are given at 10 and 16 days postpartum in milked cows, then LH rises; however, in autumn calved suckler beef cows 20 days had to elapse and in spring-calved suckler beef cows 30 days had to elapse. Further evidence of the refractory state of the hypothalamus and pituitary gland has been demonstrated by the failure of a 1 mg dose of oestradiol benzoate to elicit a surge of LH at 0–5 days postpartum; a response was obtained by 10 days which was increased by 25 days. A dominant follicle may emerge from the first follicular wave, but ovulation will occur only if the dominant follicle produces enough oestradiol to stimulate adequate LH secretion in the form of one pulse per hour; if this occurs, then there is a first ovulation at 21 days in dairy and 31 days in beef cattle. Insulin growth factor (IGF-1) is also involved in the early onset of folliculogenesis and ovulation, by stimulating follicular granulosa cell aromatase activity and oestradiol synthesis. After ovulation, there is a luteal phase which may be of normal length with a return to oestrus after 18–24 days, or it may be much shorter, 14 days or less; the latter occurred in 25% of dairy but in 78% of beef cattle. These short luteal phases probably arise because of
inadequate preovulatory development of the follicle so that it either becomes luteinised in the absence of ovulation, or more likely luteinisation of the CL is inadequate. These short luteal phases are more prevalent the earlier the return of normal ovarian activity, i.e. 100% at 0–5 days, 60% at 10–15 days and 10% at 25–30 days postpartum. However, it is now accepted that the first sign of oestrus is not always a true reflection of the onset of cyclical activity. This is because the CNS requires prior exposure to progesterone to elicit behavioural signs; a similar phenomenon occurs in ewes at the beginning of the breeding season. Following calving, the reproductive strategy of the cow is transformed from delivering and nourishing a healthy calf to reestablishing pregnancy. The dormancy of ovarian follicular development that prevailed during late pregnancy must now be replaced by a sequence of events culminating with behavioral oestrus, ovulation of healthy follicles and normal luteal function. These are the requirements for successful reproductive performance in any type of cattle production system.

**Establishment of the First Ovulation Postpartum:**

A period of anovulatory anestrus of varying duration is observed in both milked and suckled cows following parturition. In milked dairy cattle the interval from calving to first ovulation is typically between 19 and 22 d. Under pasture-based management systems the mean interval is 43 d and in suckled cows it may vary between 20 and 86 d. In pasture-based dairy herds, between 13 and 48% of cows were diagnosed as anovulatory anestrus 1 wk before the start of the breeding period. In suckled beef herds, an average of 23% of cows had not ovulated by the start of breeding, with the percentage increasing by 6 percentage units for each 10 d decrease in interval from calving. Concentrations of gonadotropins are very low in late pregnancy due to strong negative feedback from progesterone and estrogens. After calving, concentrations of FSH increase within 5 to 10 d in both milked and suckled cows, whereas circulating concentrations of LH generally start to increase between 10 to 20 d postpartum. Pulsatile episodes of LH release are first detected around this time in milked cows, but are delayed in suckled cows, with frequency of pulses of LH release being correlated with the interval to first ovulation. A single large, or dominant, ovarian follicle commences growth around 10 to 14 d postpartum in both milked and suckled cows. This first dominant follicle may fully mature and ovulate, or become atretic and be replaced by one or more subsequent dominant follicles, or may continue growth and become cyclic. Ovulation of a dominant follicle occurs when production of estradiol by the follicle is sufficient to stimulate a preovulatory surge of LH and FSH. Estradiol production is in turn dependent on sufficient gonadotropin support in terms of LH pulse frequency and increased plasma concentrations of estradiol are associated with elevated plasma concentrations of IGF-I. Both IGF-I and insulin are potent stimulators of steroidogenesis and proliferation of bovine granulosa and theca cells in vitro, acting synergistically with FSH or LH. The first postpartum ovulation is frequently associated with an absence of estrous behavior and is often followed by a luteal phase of short duration. The short luteal phase following the first postpartum ovulation is a consequence of interactions between the uterus, the corpus luteum, and possibly the ovulatory follicle. Premature release of PGF2α by the uterus, rather than inadequate luteal development, is the main cause of the shortened life span of the first corpus luteum. Low or negligible concentrations of progesterone preceding the first postpartum ovulation result in lower numbers of progesterone receptors and greater numbers of oxytocin receptors in endometrial cells, allowing early development of the positive feedback loop between oxytocin and PGF2α. Low preovulatory concentrations of estradiol are also probably involved in increasing the numbers of
endometrial oxytocin receptors, thus allowing binding of oxytocin and premature release of luteolytic PGF2α. A short period of elevated progesterone concentrations during the postpartum period, from either endogenous or exogenous sources, is important for the expression of estrus as well as subsequently normal luteal function. The mechanism of action is not clear, but it appears to involve changes in estradiol receptor number in the hypothalamus and increases in estradiol production. Treatment of anestrous cows with progesterone results in greater follicular fluid and circulating concentrations of estradiol, increased pulsatile release of LH and increased numbers of receptors for LH in granulosa and theca cells in preovulatory follicles, compared with untreated animals. It is hypothesized that exposure of anestrous cows to progesterone may stimulate development and maturation of a dominant follicle by enhancing release of LH and stimulating development of LH receptors and secretion of estradiol. The increased release of LH is possibly due to a reduction in estradiol receptors in the hypothalamus and reduced negative feedback on release of GnRH, as demonstrated in the prepubertal heifer. The interval from calving to first postpartum ovulation is characterized by a period of increasing pulsatile release of LH, associated with the growth and development of ovarian follicles. In order for these follicles to mature and ovulate, gonadotropic support must be sufficient to stimulate increased production of estradiol, which can induce a preovulatory surge of LH and FSH. In addition, circulating concentrations of metabolic hormones, such as IGF-I, are involved in optimizing the response of ovarian granulosa and theca cells to gonadotropin stimulation. The luteal phase following the first postpartum ovulation is of short duration, due to premature release of PGF2α from the uterus, associated with greater numbers of oxytocin receptors in the endometrium. This short period of elevated progesterone concentrations is required for the full expression of estrus and a luteal phase of normal duration.

**Establishment of the First Normal-Length Luteal Phase**

The first postpartum ovulation is frequently associated with an absence of estrous behavior and is often followed by a luteal phase of short duration. The short luteal phase following the first postpartum ovulation is a consequence of interactions between the uterus, the corpus luteum, and possibly the ovulatory follicle. Premature release of PGF2α by the uterus, rather than inadequate luteal development, is the main cause of the shortened life span of the first corpus luteum. Low or negligible concentrations of progesterone preceding the first postpartum ovulation result in lower numbers of progesterone receptors and greater numbers of oxytocin receptors in endometrial cells, allowing early development of the positive feedback loop between oxytocin and PGF2α. Low preovulatory concentrations of estradiol are also probably involved in increasing the numbers of endometrial oxytocin receptors, thus allowing binding of oxytocin and premature release of luteolytic PGF2α. A short period of elevated progesterone concentrations during the postpartum period, from either endogenous or exogenous sources, is important for the expression of estrus as well as subsequently normal luteal function. The mechanism of action is not clear, but it appears to involve changes in estradiol receptor number in the hypothalamus and increases in estradiol production. Treatment of anestrous cows with progesterone results in greater follicular fluid and circulating concentrations of estradiol, increased pulsatile release of LH and increased numbers of receptors for LH in granulosa and theca cells in preovulatory follicles, compared with untreated animals. It is hypothesized that exposure of anestrous cows to progesterone may stimulate development and maturation of a dominant follicle by enhancing release of LH and stimulating development of LH receptors and secretion of estradiol. The increased
release of LH is possibly due to a reduction in estradiol receptors in the hypothalamus and reduced negative feedback on release of GnRH, as demonstrated in the prepubertal heifer. To summarize, the interval from calving to first postpartum ovulation is characterized by a period of increasing pulsatile release of LH, associated with the growth and development of ovarian follicles. In order for those follicles to mature and ovulate, gonadotrophic support must be sufficient to stimulate increased production of estradiol, which can induce a preovulatory surge of LH and FSH. In addition, circulating concentrations of metabolic hormones, such as IGF-I, are involved in optimizing the response of ovarian granulosa and theca cells to gonadotropin stimulation. The luteal phase following the first postpartum ovulation is of short duration, due to premature release of PGF2α from the uterus, associated with greater numbers of oxytocin receptors in the endometrium. This short period of elevated progesterone concentrations is required for the full expression of estrus and a luteal phase of normal duration.

The major causes of true anoestrus characterized by lack of ovulation are:

1. In heifers, bilateral gonadal aplasia, which causes permanent anoestrus and sterility, occurs in the common condition of freemartinism, found in the majority of female calves that are cotwins with males. It is also observed in bilateral ovarian hypoplasia, a rare inherited condition, in which the genes are passed on through fertile individuals with unilateral gonadal hypoplasia.

2. Cows a variety of abnormalities at or around calving, such as dystokia, retained fetal membranes, hypocalcaemia, metritis, endometritis and other postpartum abnormalities may result in delayed return to cyclicity.

3. Ovarian cysts, traditionally defined as fluid-filled structures >25mm in diameter and present for more than 90 days in the absence of a corpus luteum (CL), are quite commonly recorded in cases of anoestrus and in fact the majority of cows with cystic ovarian disease exhibit anoestrus. This traditional definition implied that cysts were static structures; however, this is now known not to be the case, and cysts should be considered dynamic both in structure and function. The likely hormonal mechanism of cystic ovarian disease is failure of the preovulatory luteinizing hormone (LH) surge. In some cases there may be failure of the ovary to respond to LH at the start of oestrus. Cystic ovarian disease is a very complex condition and in many cases the cyst produces a mixture of steroids, which are variably absorbed into the general circulation. In the postpartum period, particularly during the first six weeks, some cows (approximately 20–25 per cent) experience ovulation failure followed by the development of palpable but functionally transient cystic structures that may not interfere with subsequent cyclicity. Towards the end of this period many farmers become concerned about lack of oestrus and so the cow is presented for veterinary examination. Cystic ovaries were associated with 6 to 11 more days to first service and with 20 to 30 more days to conception. By about six weeks after calving an ovarian cystic structure should be regarded as pathological. Cysts are often classified as either follicular (about 70 per cent; single large cyst or multiple smaller cystic structures) or luteal and it is easy to understand why the cow with the luteal cyst, which is predominantly progesterone producing, does not exhibit oestrus, though it is less clear why she fails to cycle. However, follicular cysts with oestrogeon production are also found in cases of anoestrus. This may be due to the low level of the steroid produced in some cases, while in other cases it may result from the negative effect on the behavioural centre of high levels of
oestrogen circulating more or less continuously. Another explanation for the lack of oestrus in cows with follicular cysts is that the behavioural centres in the brain, which control the expression of oestrus, respond better to oestrogens if they have been primed by progesterone.

4) The role of nutritional deficiencies in anoestrus is relatively straightforward in heifers but is complex in adult dairy cows.

5) Among miscellaneous causes of persistent corpus luteum is the rare condition of uterus unicornis, in which one horn of the uterus is absent. Ovulation on the side without a uterine horn results in a corpus luteum that is not exposed to locally produced prostaglandin and therefore does not regress after 16 or 17 days.

Silent ovulation and unobserved oestrus

First ovulation postpartum is generally silent (80 per cent), but once cycles have been established, silent oestrus is not a common phenomenon. However, ovulation without the accompanying signs of oestrus (that is, silent oestrus) does occur. The best documented situation relates to the first ovulation after calving, particularly if it occurs within the first 20 days. Otherwise it is almost impossible to distinguish between silent and unobserved oestrus. Some instances of oestrus are so weak or last for such a short time that detection by conventional observation is a matter of chance. Standing oestrus lasts for about 8–12 hours in cows and 6–8 hours in maiden heifers. However, 20 per cent of heats last less than 6 hours.

Diagnosis

Stage 1: assessment of ovarian status

Since the word ‘cycle’ implies repetition, theory would dictate that assessment of whether or not a cow is cycling necessitates repeated examinations over an interval that should allow the observation of two oestrous periods. Under practical conditions it is usually assumed that the animal is cycling if it is possible to prove that ovulation has recently taken place. Another common assumption concerns a group of animals. It is usually considered that the group as a whole is cycling if two-thirds of the cows are in the luteal phase, i.e. the same fraction of the oestrous cycle that the luteal phase occupies.

History taking for anoestrous cows.

• Date of calving
• Any abnormality around after calving
• Has the cow been seen in oestrus since calving?
• Could the cow be pregnant?
• Oestrous detection methods in the herd
• Are there other cows in the herd with similar problems?

The next step is to find out if there is functional luteal tissue present in the ovaries. The traditional way to do this is to palpate the ovaries per rectum, but there are now several choices. One is to carry out a single or a series of milk progesterone assays, which gives a quick, reliable result. Another is ultrasound scanning, which allows the user to observe functional luteal tissue in the ovary.

Likely reasons for the presence of functional luteal tissue.

• Cycling (days 5–17 inclusive)
• Pregnancy
• Ovarian pathology (luteal cyst)
• Uterine pathology (pyometra with persisting corpus luteum)

Assessment of the cause of anoestrus or silent ovulation

Causes of dysfunction of the ovarian cycle
• Freemartinism (congenital)
• Ovarian hypoplasia (inherited)
• Uterus unicornis (congenital)
• Pyometra (periparturient problems)
• Some cases of inactive ovaries
• Nutritional and metabolic stress
• Systemic disease
• Delayed involution

In cases of delayed involution, the enlarged uterus and particularly the enlarged cervix, which involutes more slowly, usually make the diagnosis of the immediate cause easy. However, on some occasions, by the time the cow is examined the uterus may well have returned to normal size and the veterinarian will have to depend on a history of periparturient problems and possibly of abnormal vaginal discharges noted by the farmer. The clinician should look for any other disease condition in the cow examined for anoestrus. Especially important is any disease that causes loss of body condition or pain.

The veterinarian should find out about the following:
• Organization of oestrous detection.
• Ease of cow identification.
• Method of recording.
• Layout of the buildings.
• State of the floors and levels of lighting.
• The way the cows are handled, moved around and assigned to groups.
• Amount of knowledge of oestrus and the oestrous cycle among the people who are detecting oestrus.
• Motivation for oestrous detection.

Factors likely to prevent postpartum anoestrus
• Appropriate body condition and nutrition in the dry period
• Normal calving
• Disease-free puerperium, or adequate rapid treatment of diseases in the puerperium
• Suitable transition period feeding and regular checking of body condition score postpartum
• Efficient oestrous detection

TREATMENTS TO REDUCE THE POSTPARTUM INTERVAL:
Treatments options for cows with an extended PPI may be categorized into management tools and hormonal interventions. The former may include nutritional supplements and reduced milking frequency (either calf removal or once daily milking of dairy cows). Hormonal interventions aim to induce ovulation and estrus by stimulating maturation of ovarian follicles, by directly or indirectly inducing a surge in release of LH.

HORMONAL TREATMENTS
Ovulation can be successfully induced in anestrous beef or dairy cattle simply by using GnRH analogues or chorionic gonadotropins. However when used in isolation, such treatments require the presence of a functional ovarian dominant follicle, they are not always associated with expression of estrus and a majority of animals return to anovulatory anoestrus following treatment. Estradiol may also be used to induce estrus with or without concurrent ovulation. With all these treatments, ovulation is usually followed by a luteal phase of short duration, but when preceded by a period of
treatment with progesterone or a progestogen they are usually followed by normal length estrous cycles. Therefore, most hormonal treatments involve the use of progesterone or a progestogen. The following section describes a number of different protocols, which either have been evaluated in controlled trials in anestrous suckled beef cows or milked dairy cows. The use of progesterone alone to stimulate the early resumption of estrous cycles in suckled or dairy cows was reported to be successful in some studies. The development of controlled intravaginal progesterone releasing devices such as the PRID (progesterone-releasing intravaginal device) and CIDR (controlled internal drug releasing device) allowed the development of long-term hormonal treatments, which minimized the requirement for repeated handling of animals and circumvented delivery problems associated with the feeding of oral progestogens or injecting progesterone. Treatment of suckled beef cows with CIDR devices at the time of restricted calf suckling did not reduce the interval to first ovulation, but significantly increased the duration of the post treatment interovulatory interval, as well as increasing luteal function and size. Use of a CIDR device for 7 d and PGF2α on 6 d after insertion, at the start of the breeding period in anestrous suckled beef cows, effectively reduced the interval to first estrus compared with untreated controls (8 vs. 11 d), but did not influence pregnancy rates over a 31-d breeding period. In anestrous dairy cows, treatment with CIDR devices for 7 d following an injection of PGF2α at 12 to 14 d after calving significantly increased the percentage cows displaying estrus by 30 d after calving and reduced the interval to first luteal activity, compared with untreated controls. Thus, the use of progesterone alone may be beneficial for initiating normal length estrous cycles and may have a synchrony effect when used with PGF2α, but the response is variable and does not include synchronization of ovulation. Treatment with a chorionic gonadotropin after a period of progesterone treatment, with the aim of stimulating ovarian follicular development and production of estradiol, has been evaluated in both suckled and milked cows, although the results obtained have been variable. The development of treatment regimens using a CIDR device with equine chorionic gonadotropin (eCG) injected at the time of device removal. Therefore, eCG may be used following a period of progesterone treatment to induce estrus and ovulation in anovulatory anestrous cows, but this protocol does not result in a consistent improvement in reproductive performance. It has largely been replaced by regimens including estradiol. Estradiol has been used to stimulate ovulation and expression of estrus following progesterone treatment in a number of studies. In early studies in beef cattle, injections of progesterone for 9 to 14 d followed by a single injection of estradiol successfully induced estrus and ovulation and reduced the interval to conception, compared with untreated controls. Use of an oral progestogen followed by an injection of 5 mg of estradiol valerate significantly reduced the interval to first estrus and ovulation; although conception rates to first service were less compared with those of untreated cows. Studies using CIDR devices for 7 d with an injection of 1 mg of estradiol benzoate (EB) given 24 to 30 h after device removal demonstrated a significant improvement in the percentage of anestrous beef cows displaying estrus and forming corpora lutea with a normal lifespan, as well as conceiving to insemination, compared with treating cows with progesterone alone or no treatment. Treatment of anovulatory anestrous cows with CIDR devices for 6 d, followed by an injection of 1 mg of EB 24 h after device removal, typically results in 87% of cows being detected in estrus within 7 d of EB injection (varying from 69 to 100% among herds) and 42% of cows conceiving to insemination during this period (varying from 27 to 62%). It has been speculated that treatments to induce emergence of a new
ovarian follicular wave at the start of progesterone treatment may improve fertility following insemination at the induced estrus. In cows that have resumed estrous cycles, prolonged periods of treatment with low concentrations of progesterone result in the development of persistent ovarian follicles (1993), that are associated with reduced fertility. In these cows, regression of a dominant follicle and synchronous emergence of a new follicle wave may be induced by treatment with GnRH analogues or EB. However, in anestrous anovulatory dairy cows, use of EB at the time of CIDR device insertion, either in the form of a 10-mg intravaginal capsule, or an i.m. injection of 0.5 or 1 mg of EB did not significantly influence the percentage of cows displaying estrus or conceiving to first insemination after CIDR device removal.

Gonadotropin-releasing hormone analogues may also be used at the start of progesterone treatment to regress the dominant ovarian follicle present and synchronize emergence of a new cohort of follicles. This protocol has the additional effect of inducing ovulation and the formation of a corpus luteum in a majority of cows, resulting in elevated concentrations of progesterone in plasma compared with cows not treated with GnRH. To ensure the absence of luteal tissue following progesterone device removal, PGF2α is generally included in such protocols. In field trials conducted in pasture-based dairy herds, cows were treated with the GnRH analogue, buserelin, at the start of a 6-, 7- or 8-d period of CIDR device insertion, with PGF2α at device removal and 1 mg of EB 24 h later, compared with treatment with a CIDR device and EB alone. Conception rates to first insemination were increased and interval to conception decreased by inclusion of GnRH. In anestrous beef cows treated with GnRH and a norgestomet implant for 7 d and PGF2α injected at implant removal, nearly 60% of treated cows were detected in estrus within 6 d after PGF2α and pregnancy rates in this period were significantly greater as compared with cows treated with two injections of PGF2α, 14 d apart. Treatment with GnRH followed by PGF2α 7 d later and GnRH 9 d later, with a CIDR device inserted for 7 d after the first GnRH improved pregnancy rates in anestrous cows compared with animals treated with GnRH, PGF2α and GnRH. Thus, treatment regimens including a GnRH agonist at the commencement of progesterone treatment, and PGF2α at the end, produce good responses in anestrous beef and dairy cattle, with results comparable with, or better than, those obtained with other hormonal protocols. Programs in combination with PGF2α to initiate resumption of estrous cycles, without the requirement for exogenous progesterone treatments. A protocol developed for use in dairy cows that have resumed estrous cycles involves the sequential injection of GnRH, PGF2α and GnRH at intervals of 7 and 2 or 2.5 d, respectively, with all treated cows being inseminated 16 to 24 h after the final injection of GnRH, without estrous detection. Treatment of cows that have not been detected in estrus, but have a detectable corpus luteum, has mainly focused on the use of PGF2α. In cows with an ultrasonographically detectable corpus luteum, treatment with PGF2α resulted in 55% of animals being detected in estrus within 6 d of treatment. When the corpus luteum was detected following palpation of the ovaries, estrous response rates within 6 d of treatment averaged 52% and varied from 36 to 68%, with the majority of cows displaying estrus within 4 d of treatment. These results demonstrate that the estrous response to PGF2α treatment in this class of cow is not great compared with that observed in cows that have resumed estrous cycles, with between 63 to 88% of cows being detected in estrus within 7 d of treatment with PGF2α. The use of PGF2α was compared with an Ovsynch protocol in French dairy cows that had not been detected in estrus but had a palpable corpus luteum. Pregnancy rates by 50 d after the start of treatment were similar in the two treatment groups, indicating that the Ovsynch
protocol may be used to treat ‘subestrous’ cows, with the benefit of no requirement for estrous detection. Other studies have included progesterone in treatment protocols with the aim of improving pregnancy rates and reducing the interval to conception in this class of cow. An alternative protocol, which has also been evaluated in commercial herds, is to treat cows with a GnRH analogue at the time of insertion of a CIDR device, with PGF2α at device removal (7 d later), followed by 1 mg of EB 2 d later. This protocol significantly increased submission rates and pregnancy rates in the first 21 d of breeding compared with untreated cows.

Factors Affecting Response to Hormonal Treatments

Hormonal treatments can effectively reduce the interval to first ovulation, and synchronize estrus, across cows in a variety of physiological states. However, responses to treatments are not uniform either across herds or across cows within herds and appear to be dependent on those factors influencing the prevalence of anestrus, such as age, body condition, and interval from calving. Younger cows have a lower probability of being detected in estrus after treatment and body condition influences pregnancy rates following treatment in both suckled beef and dairy cattle. Mobilization of body fat, was also reported to be higher in suckled beef cows not conceiving compared with those that did conceive following treatment with norgestomet and eCG. Interval from calving to treatment is positively associated with the percentage of cows ovulating following treatment, as well as pregnancy rate following treatment. Cows that are in poor body condition or have recently calved may not ovulate following treatment, or may display estrus without ovulating and return to anestrus. The latter group of animals may be assumed to be pregnant, having displayed estrus once and not returned to estrus. At present it is not possible to easily determine which cows have responded and conceived, rather than displayed estrus but not ovulated or conceived, before 30 d after insemination, when ultrasonographic pregnancy diagnosis can be conducted. In some situations, monitoring milk progesterone concentrations at, say, 14 and/or 21 d after treatment may be beneficial, especially where ‘Cowside’ test kits are available.

Suboestrus or silent heat

A number of authors have shown that the first and second ovulations postpartum frequently are not accompanied by behavioural signs of oestrus, and are thus truly ‘silent heats’. It is, however, unlikely that many true ‘silent heats’ occur after the second cycle. When ovulation occurs in the absence of observed oestrus it is more likely to be the result of a failure of observation than of an impaired expression of oestrous behaviour. A genetic predisposition to silent heat has been identified, with certain sire lines showing a statistically significant effect. The same authors found that it was more common in the hotter months of the year, although in temperate climates it has been shown to be more common in the winter than in the summer months. Suboestrus has undoubtedly been associated with heat stress, whilst cows suffering from ‘fescue toxicity’ (ergotism) may also become suboestrous. A number of nutritional deficiencies are also said to cause suboestrus, including β-carotene, phosphorus, copper and cobalt. Attempts have been made to identify an endocrinological reason for a cow failing to show behavioural signs but to date none has been identified.

Diagnosis silent heat

Diagnosis of the condition is made on the clinical history and rectal palpation of the genital system. No differentiation can be made from nonobserved oestrus, since the clinician will be checking for evidence of cyclical ovarian activity as demonstrated by the presence of a corpus luteum or (if the cow is in late dioestrus, early dioestrus or
oestrus), by the presence of good uterine tone. The corpus luteum must be differentiated from a cyst; it may be persistent or the cow may be pregnant. If there is any doubt, then a re-examination should be made in 10 days. Since the accuracy of identifying a corpus luteum by rectal palpation has been reported as 89% and 77%, the determination of progesterone in milk or blood, or the use of transrectal ultrasonography, should confirm its presence.

**Treatment**

Treatment is simple. If a mature corpus luteum is present and the cow is not pregnant, PGF2α or an analogue can be given, followed by fixed-time insemination or breeding at the time of observed estrus.

**Persistent corpus luteum**

Anything, which interferes with the production or release of PGF2α, will result in a persistent corpus luteum. The presence of uterine infection and inflammation of the tissues, there is interference with the production or the release of PGF2α. This condition can be self-perpetuating since progesterone domination of the uterus reduces its resistance to infection and prevents recurrent periods of estrus when the uterus is more resistant. One consequence of this is pyometra that, if untreated, can persist for several months. It is considered that a persistent corpus luteum is present, it can be readily treated with PGF2α or a synthetic analogue, provided, of course, that the clinician is confident that the cow is not pregnant.

**Anoestrus in the mare**

**MARES THAT FAIL TO CYCLE**

**Causes:**

- Seasonality
- Spontaneous prolongation of the corpus luteum
- Behavioral anestrus
- Lactational anestrus
- Tumors
- An ovulatory follicles
- Developmental abnormalities

**Winter anoestrus**

The majority of mares kept out of doors will enter winter anoestrus, with the exception of approximately 30% of native pony mares. Mares are considered to be sexually inactive during winter anoestrus, however an uncommon observation in some is apparent oestrous behaviour, whilst others may have mammary enlargement and production of a milk-like substance. The lack of gonadotrophin stimulation results in small inactive ovaries that are normally smooth and firm in texture. Often the ovulation fossa is not palpable. The uterus becomes small and atonic, and at biopsy there is glandular atrophy.

**Endocrinologically, anoestrus is typified by:**

- Baseline concentrations of plasma LH;
- Random fluctuations of plasma FSH (absolute concentrations may be high due to lack of feedback from ovarian oestrogen and inhibin);
- Baseline concentrations of oestrogen;
- Baseline concentrations of progesterone.

**Behaviourally, anoestrus is typified by:**

- Lack of cyclical changes in behaviour;
- Disinterest or slight resistance to the stallion;
• Disinterest in other mares.

**SEASONALITY**

Seasonal effects on the reproductive cycle are a common cause for failure to cycle or abnormal cycles in the winter and spring. In the winter the content and secretion of the GnRH from the hypothalamus is drastically reduced. Shortly after the winter solstice GnRH secretion begins to increase through mechanisms not completely understood. Follicular Stimulating Hormone increases presumably in response to the increase in GnRH and follicles begin to develop on the ovaries. During this time little if any LH is secreted as the gene for LH synthesis is essentially turned off. The pattern of follicular growth during vernal transition is relatively predictable, with increased size and numbers of follicles. Of major importance, the first several follicles that form in vernal transition do not ovulate, although they may reach normal preovulatory size (>30 mm). Mares make on average 3.7 ± 0.9 follicles that reach a size of 30 mm or greater that do not ovulate during the transitional phase. These follicles are not steroidogenically competent and do not produce estrogen. This leads to reproductive inefficiency as it is difficult to know whether a given follicle is competent and will it ovulate. Monitoring development of a follicle over time may be useful in determining the eventual status and outcome of a transitional follicle because the growth rate of follicles destined to regress is considerably slower than that of the follicle which eventually ovulates. Shedding of the long winter hair coat in spring is a rough indicator of impending ovulation as shedding is closely associated with reproductive renewal. While it is not known what factors contribute to the eventual development of the first competent follicle, it is clear that this follicle, destined to be the first to ovulate in the year, is steroidogenically competent. The first ovulatory follicle of the year is accompanied by a surge of plasma estradiol that is followed by a surge in LH.

**Spring transitional phase**

The transitional phase is a slow period of change from inactivity to the return of normal cyclical activity and may last up to six weeks. The period is often characterised by persistent or irregular oestrous activity. Many owners become

<table>
<thead>
<tr>
<th>Ovaries</th>
<th>Season</th>
<th>Tubular Tract</th>
<th>Behavior</th>
<th>Other Findings</th>
<th>Probable Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal size; CL; follicles</td>
<td>Summer</td>
<td>Excellent tone</td>
<td>Rejects stallion</td>
<td>P4 &gt; 2 ng/ml</td>
<td>Prolonged diestrus</td>
</tr>
<tr>
<td>Normal size active</td>
<td>Spring</td>
<td>Lacks tone</td>
<td>Passive or irregular estrus</td>
<td>Spec: C-V pale, dry; P4 &lt; 1.0 ng/ml</td>
<td>Transition from anestrus to ovulatory stage</td>
</tr>
<tr>
<td>Small inactive</td>
<td>Winter</td>
<td>Flaccid, open</td>
<td>Passive or irregular estrus</td>
<td>Spec: C-V pale, dry</td>
<td>Winter anestrus</td>
</tr>
<tr>
<td>Very small inactive</td>
<td>Any</td>
<td>Uterus infertile</td>
<td>Passive</td>
<td>Small body size</td>
<td>Gonadal dysgenesis</td>
</tr>
<tr>
<td>One ovary enlarged</td>
<td>Any</td>
<td>Variable (flaccid)</td>
<td>Masculine aggressive, irregular constant estrus, anestrus</td>
<td>No ovulation fossa; cystic appearance; Inhibit increased if GCT</td>
<td>Secrating ovarian tumor</td>
</tr>
<tr>
<td>Other one smaller than normal</td>
<td>Any</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

Abbreviations: C-V: cervix, vagina; P4: progesterone; GCT: Granulosa Cell Tumor
frustrated, since they wish to breed the mare and achieve an early foal the next year. Early in the transitional phase there is moderate follicular development; later there may be exuberant growth of follicles, such that each ovary can be almost twice the size found during the ovulatory phase. Many follicles up to 15 mm in diameter may be present. Later, there may be many follicles greater than 35 mm. In these cases ovaries are large, with palpable follicles. Transitional follicles do not ovulate (possibly as a result of a failure of LH synthesis). The lack of oestrogen means there are few oestrogenic effects on the reproductive tract (unlike the situation during true oestrus).

**Endocrinologically, the transitional phase is typified by:**
- Increased amplitude and frequency of GnRH release;
- Increased FSH early in the transitional phase, with decreasing concentrations one to two weeks before the first ovulation;
- Slowly-increasing concentrations of LH with a rapid increase before the first oestrum and a peak just after the first ovulation;
- Relatively low concentrations of oestrogen that increase with the follicle wave prior to the first ovulation;
- Low concentrations of progesterone until after the first ovulation.

**Behaviourally, the transitional phase is typified by:**
- Variable signs between and within mares;
- Some mares have poor signs of oestrus, others have persistent oestrus;
- Many mares show erratic signs of oestrus. Ultimately, one follicle wave (usually associated with greater oestrogen production and significant oestrogenic effects on the reproductive tract) ovulates and the mare enters the first luteal phase of the year.

**OVARIAN TUMORS**
Granulosa-theca cell tumors are the most commonly reported neoplasm of the equine ovary. They are generally benign, steroid-producing neoplasms with variable effects on mare behavior, depending upon the hormone levels attained. Mares may exhibit anoestrus, continuous or intermittent oestrus, or stallion-like behavior, including mounting mares in oestrus, aggressiveness, squealing and striking. Granulosa cell tumors are almost always unilateral, slow growing and benign.

**Diagnosis of Anoestrus**

**Rectal palpation**
- Ovaries: small, hard, bean shaped; may have small follicles.
- Uterus: thin walled and flaccid, may be difficult to palpate.
- Cervix: soft and indistinct.

**Visual examination of the vagina**
- Vaginal wall is pale (blanched) and dry.
- Cervix is flaccid and atonic; closed but may gape open to reveal uterine lumen.

**Palpation per vaginam**
- Vagina is dry.
- Cervix is dry, short and easily admits two fingers but may be tighter in maiden and older mares.

**Ultrasound examination**
- Ovaries: small, multiple small follicles (less than 1.5 cm diameter); no luteal tissue.
- Uterus: small, homogeneous, hypoechoic appearance.

**Diagnosis of Transitional phase**

**Rectal palpation**
- Ovaries: become softer, follicles grow (3–5 cm) and then regress. May be repeated waves of follicular growth and regression. Difficult to anticipate which follicle will
grow to ovulatory size and when. Ovulation usually followed by regular ovulatory cycles.

- **Uterus**: flaccid or slight tone, may be similar to oestrus.
- **Cervix**: remains soft and difficult to palpate per rectum.

**Visual examination of the vagina**

- Vaginal-wall appearance depends upon ovarian function, i.e. from typically anoestrus to typically oestrus.
- Cervix frequently appears similar to early oestrus.

**Palpation per vaginam**

Vagina and cervix frequently feel similar to early estrus.

**Ultrasound examination**

- Ovaries: large, contain multiple follicles of varying sizes. In early transition, follicles may be multiple, small; later follicular size increases, and follicles may reach 5cm in diameter. Follicles regress (decrease in size) without ovulating. No luteal tissue is identified.
- Uterus: frequently oedema present, associated with growth of a specific follicle. Oedema is more variable than during estrus of the normal breeding season. Anechoic (black), free, luminal fluid may be present.

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**INFERTILITY**

The term fertility is applied to cattle denote the desire and ability to mate, the capacity to conceive and to nourish the embryo and finally the power to expel a normal calf and fetal membranes.

- **Fertile cow**: cows produce one viable calf each year.
- **Sterility**: an absolute inability to reproduce.
- **Infertility**: degree of reduced fertility.

**ANATOMICAL FACTORS AFFECTING FERTILITY**

1. **Congenital anomalies**
   - Ovaria agenesis or ovarian aplasia
     - Infantile genital tract and absence of cyclic behavior, hereditary condition.
   - Ovarian hypoplasia
     - Ovary: Small, narrow, functionless, furrowed, spindle like.
     - Genital tract infantile no estrous cycles
     - Inherited or autosomal recessive gene.
     - Differential diagnosis: Functional aneestrous.

2. **Intersexuality or freemartin**
   - Origin-Anastomosis of blood vessels of the adjacent allantoic sac of heifer-calf born as co-twins. This anastomosis allows male hormone from earlier differentiating fetal testicles to retard the development of the undifferentiated gonad and Mullerian system and to stimulate its Wolfian duct. The result is intersex with outward appearance of female and internal sex organs of mixed type. The ovaries remain hypoplastic. The vulva showed a prominent tuft of hair from the inferior commissure and clitoris is
markedly enlarged. Cervix is absent and uterus is represented by two solid cords and at the neck of the bladder two well-formed seminal vesicles.

**Diagnosis:**

1. History; co-twins male and female.
2. Permanent clitoris and hair tuft
3. Lack of vaginal patency; using vaginal speculum
4. Anestrous.
5. Rectal palpation; vestigial female reproductive organs and rudimentary male organs.
6. Segmental aplasia of the Mullerian ducts "White heifer disease"
   Leads to various anomalies of the vagina cervix and uterus.
   Ovaries develop normally with normal cyclical behavior.

a. Persistence of hymen;
   Vaginal constriction in front of urinary meatus or as partition with central aperture or complete partition between vulva and vagina this type usually discovered during parturition causing dystocia.

b. Hymen obstruction is complete;
   Discovered during insemination
   Accumulation of cyclic secretions in front the obstruction
   May become infected during insemination and become infected during insemination and become pyogenic secretion- Fever, shivering.
   Treatment: Trocar and cannula.

**HEREDITARY ORIGIN**

c. Segmental aplasia
   Vagina, cervix, uterus OVARIES ARE NORMAL
   UTERUS UNICORNIS; one uterine horn
   UTERUS DIDELPHYS; each uterine horn connect with the vagina by separate cervical canal causes dystocia due to Sex-linked recessive gene with linkage to the gene for white color coat. (Inbreeding.)

5. Aplasia of oviduct
6. Paraovarian cyst
   Commonly about 1cm in diameter with thick mucoid fluid present in bursa and mesosalpinx. Some tissues impinge on oviduct and reduce its lumen causing oviduct obstruction.

7. Atresia of vulva
   Hereditary origin.

**Female genital tract infection:**

Fertility is one of the key determinants of the lifetime performance of a cow. For beef cows and for pastoral dairy cows, it is necessary for a calf to be produced every 365 days. For intensively managed dairy cows, the need to produce a calf each year is less of a vital; yet even for these animals, regular calving is still essential for the establishment of lactations. Regular breeding depends upon the normal function of the reproductive system. In order to breed regularly, the cow has to have functional ovaries, display estrous behaviour, mate, conceive, sustain the embryo through gestation, calve, and resume estrous cyclicity and restore uterine function after calving. Each of these aspects of reproductive function can be affected by management, disease and the genetic make-up of the animal. When the function of the reproductive system is impaired, cows fail to produce a calf regularly. When this occurs, the term ‘sterility’ is used to mean an absolute inability to reproduce; whereas the term ‘infertility’ either is considered to be synonymous with sterility, or may
imply a delayed or irregular production of the annual live calf. The term ‘subfertility’ is probably a more appropriate term for the latter. Both congenital and acquired abnormalities of the genital system can influence fertility.

One of the most significant causes of infertility in cattle is the complex of diseases that includes retained fetal membranes (RFM), puerperal metritis, endometritis, pyometra and other nonspecific infections of the uterus. These diseases share common etiological factors, predispose to one another and, to a large extent, share common treatments. A degree of bacterial contamination of the uterus almost always occurs during, or immediately after, parturition. Bacterial contamination of the uterus may also occur during coitus or insemination. Whether or not a persistent infection of the uterus becomes established depends upon the level of contamination, the animal’s uterine defence mechanisms and the presence of substrates (such as devitalised tissue) for the growth of bacteria. Under normal circumstances, there are several mechanisms which prevent opportunistic pathogens from colonising the genital tract. Firstly, the uterus is protected by the physical barriers of the vulval sphincter and cervix. It should be noted that, although the vulva may appear of little consequence as a barrier, it is, in fact, remarkably efficient at preventing faecal contamination of the tubular genitalia. Secondly, the uterus is protected by local and systemic defence mechanisms; both are influenced by the reproductive steroid hormones, oestrogen and progesterone. In general, it is considered that the genital tract is more resistant to infection when it is under oestrogen dominance, whilst under progesterone dominance it is more susceptible. The reproductive endocrine system therefore has a significant influence on the resistance of the genital tract to infection. It is not surprising that on the two occasions when the physical barriers are breached (i.e. at coitus, or insemination; and at the time of parturition, especially immediately postpartum) the genital tract is in its most resistant state, since it is under the dominance of oestrogens and progesterone concentrations are low. The high oestrogen concentrations that occur at oestrus and parturition cause changes in the numbers and proportions of circulating white blood cells, with a relative neutrophilia and a ‘shift to the left’. Moreover, at oestrus, the blood supply to the uterus is increased under the influence of oestrogens, whilst at parturition there is a massive blood supply to the gravid uterus. This increased blood supply, coupled with the migration of white cells from the circulation to the uterine lumen, enables vigorous and active phagocytosis of bacteria to occur. Oestrogens also cause an increase in the quantity and nature of vaginal mucus, which also plays an important role in defence of the uterus against bacteria by providing a protective physical barrier and by flushing and diluting the bacterial contaminants. Hence, despite the massive contamination with opportunistic pathogens that occurs at oestrus and parturition, the bacteria are normally eliminated quickly and there is rarely impairment of health. Since the genital tract is generally able to overcome the potential challenge of massive nonspecific bacterial contamination it is important to consider the reasons for failure. Firstly, damage to the mechanical barriers that protect the uterus makes it more vulnerable to the establishment of infection. Thus, obstetrical damage to the vulva impairs its ability to act as an effective sphincter, causing aspiration of air, ballooning of the vagina, dehydration of the mucosa and the development of vaginitis. Likewise, damage to the cervix may allow heavy contamination of the uterine lumen, especially if there is concurrent damage to the vulva. Since the main cause of both these conditions is poor obstetric practice, they should largely be preventable. It is possible to restore the barrier function of the vulva after injury or even after perineal laceration/rupture, enabling the cow to eliminate the infection. Surgical repair of the cervix is virtually impossible.
Secondly, failure of the natural defence mechanisms around the time of calving may be caused by a number of factors; these include dystocia, RFM, metabolic diseases and fatty liver disease. Injured and devitalised tissue is less resistant and is readily infected; as a result, a severe and sometimes fatal puerperal metritis can occur. Other factors which delay uterine involution have been described in Chapter 7. Finally, since progesterone domination of the genital system increases its susceptibility to infection, any condition which results in prolongation of the luteal phase can enable non-specific contaminants to become pathogenic. A persistent corpus luteum, either of dioestrus or of a degenerate pregnancy, or luteal cysts, is sometimes associated with infection of the uterus. Moreover, since infection of the uterus inevitably causes damage to the endometrial epithelium, the uterus becomes unable to secrete luteolytic patterns of PGF2α. Hence, the corpus luteum is retained and a self-perpetuating infection results.

**Classification of uterine infection:**

Several systems have been described in attempt to classify and define uterine infection. Uterine infections are generally classified according to clinical signs and degree of severity, which is in adhere to definitions used by theriogenologists.

Dohoo et al., (1984) classified uterine infection into three categories; primary metritis occurs within the first 21 days of calving, secondary metritis between 21 and 60 days, and tertiary metritis after 60 days postpartum.

Olson et al. (1986) have classified uterine infection slightly differently, puerperal metritis (acute septic metritis) occurs between the time of calving and recovery of the sensitivity of the pituitary gland to GnRH at 10-12 days postpartum. Metritis and endometritis usually occur between resolution of pituitary sensitivity to GnRH and the first postpartum ovulation. Postovulatory infections arise during the time between the first ovulation and complete uterine involution. Diseases of the postovulatory period include chronic metritis, endometritis, and pyometra.

Roberts (1986) classified endometritis into mild endometritis which is characterized by little infiltration of lymphocytes and plasma cells with few fibrosis and cystic glandular degeneration, and the second type as severe endometritis and characterized by scar tissue formation in the endometrium with thickness of uterine tissue due to high infiltration of leukocytes associated with severe changes in uterine glands including atrophy, cystic degeneration and necrosis.

Dohmen et al. (2005) classified uterine infection according to clinical symptoms and time of occurrence. Acute endometritis occurs within 14 days of parturition with two types. The first type, acute endometritis with a large amount of uterine exudates and a thin uterine wall, secondly, acute endometritis with limited amount of uterine exudates and thick uterine wall. Sub acute or chronic endometritis; occur from 14 days after parturition. Pyometra occurs from 3-4 weeks after parturition. Recently, Sheldon et al. (2005) provided a clear clinical definition of uterine diseases as toxic puerperal metritis is an acute systemic illness due to infection of the uterus with bacteria, usually within 10 days after parturition. Toxic puerperal metritis is characterized by the following clinical signs: a fetid red-brown watery uterine discharge and usually, pyrexia, reduced milk yield, dullness, inappetance or anorexia, elevated heart rate and apparent dehydration may also present. The term metritis is used for animals that are not systemically ill, but have an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina. Clinical endometritis is characterized by the presence of purulent (>50% pus) or mucopurulent (approximately 50% pus, 50% mucus) discharge detectable in the vagina after 26 days postpartum. Subclincial endometritis is defined as endometrial inflammation of the
uterus and was usually determined by cytology in the absence of purulent material in the vagina. A cow with subclinical endometritis is defined by > 18% polymorphonuclear cells in uterine cytology samples.

**Pathogenesis:**

Following calving the uterus of over 90% of all cows becomes contaminated with bacteria. Some of these bacteria are harmful and others are not. When harmful bacteria are present, the uterus may become infected. One should differentiate between uterine contamination and uterine infection. The uterus of postpartum cows is usually contaminated with a range of bacteria, but this is not consistently associated with clinical disease. Infection implies adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium, and/or release of bacterial toxins that lead to establishment of uterine disease. The development of uterine disease depends on the immune response of the cow, as well as the species and number (load or challenge) of bacteria. Number of pathogenic bacteria in the uterus of postpartum cows may be great enough to overwhelm uterine defense mechanisms and cause life threatening infection. The postpartum uterus has a disrupted surface epithelium in contact with fluid and tissue debris that can support bacterial growth. The outcome of uterine contamination depends on the number and virulence of the organisms present, as well as the condition of the uterus and its inherent defense mechanism. A mild to severe endometritis occurs in 50% of postpartum cows during the second through fourth postpartum weeks.

A variety of species of bacteria, both gram-positive and gram-negative aerobes and anaerobes, can be isolated from the early postpartum uterus. Most of these are environmental contaminants that are gradually eliminated during the first 6 weeks postpartum. A normal postpartum cow resists uterine infection by rapid involution of the uterus and cervix, discharge of uterine content, and mobilization of natural host defenses, including mucus, antibodies and phagocytic cells. Cows with certain periparturient problems have a reduced ability to control uterine infections. Excess stretching of the uterus, as with hydrops allantois, traumatization of genital tissues during dystocia or obstetric manipulation. Phagocytosis by uterine leukocytes is reduced in cow with dystocia, retained fetal membranes and metritis. If the uterus is severely debilitated, any of a variety of contaminating organisms can cause a toxic puerperal metritis. In less severe cases, an endometritis is initiated that may become persistent and impair fertility. *Arcanobacterium pyogenes* either alone or in combination with other bacteria such as the anaerobic *Fusobacterium necrophorum* and *Bacteroides spp* often is associated with uterine infections. Intra uterine oxygen reductase potential fell in the presence of infection and mostly the aerobic bacteria, thereby creating an anaerobic environment. This drop in intrauterine oxygen reductase potential may be associated with either micro-organism metabolism or increased oxygen consumption by polymorphonuclear inflammatory cells. When *A. pyogenes* was isolated from uterine fluids approximately 21 days postpartum, cows developed severe endometritis. The growth of anaerobic bacteria may enhance the establishment of *A. pyogenes* and lead to the development of severe uterine infections. Indeed, *Fusobacterium necrophorum* produce leukotoxin, while *Bacteroides* produce substances that prevent bacterial Phagocytosis and *A. pyogenes* produce a growth factor for *Fusobacterium necrophorum*.

**Uterine defense mechanism:**

Uterine defense mechanisms against contaminant micro-organisms were maintained in several ways: anatomically, by the simple or pseudostratified columnar epithelium covering the endometrium; chemically by mucus secretions from the
endometrial glands; immunologically, through the action of polymorphonuclear inflammatory cells and humoral antibodies, but the degree of interaction is not clear. Disruptions of these mechanisms allow opportunist pathogens, mostly microorganisms found in the posterior gastro-intestinal tract and around the perineal area, to colonise the endometrium and cause an endometritis. Under normal circumstances, there are several mechanisms, which prevent opportunist pathogens from colonizing the genital tract. The major anatomical barriers between the contaminated world and the relatively sterile environment of the uterus, include the vulva, the vestibule (guarded by a muscular sphincter), and the cervix. It should be noted that, although the vulva may appear of little consequences as a barrier, it is, in fact, remarkably efficient at preventing faecal contamination of the tubal genitalia. In cattle, the cervix is formidable barrier composed of series of mucosal lined collagenous rings. In addition, the cervical-vaginal mucus (especially the scant, tenacious mucus of the luteal phase) can function as a physical barrier for organisms that would otherwise ascend the reproductive tract. The circular and longitudinal layers of the uterine musculature provide physical propulsion of particular material including microbes. Epithelial cells are the first to make contact with potential pathogens that enter the uterus. Epithelial and stromal cells interactions are critically important for endometrial function, with stromal cells affecting epithelial cells through both the release of soluble factors and turns over of extracellular matrix. Conversely, epithelial cells affect stromal cells function through the release of soluble factors and cell to cell contact PGE2 regulate epithelial cells proliferation and is mediated indirectly by uterine stroma. Estradiol and progesterone have both opposing and complementary effects on the female genital tract with estradiol stimulating epithelization (especially of the vaginal lining and endometrial gland), and vascularization of the endometrium, and increased production of cervical mucus and oviductal secretions, enhancement of uterine contractility, initiation of sexual receptivity. Cattle are resistant to uterine infections when progesterone concentrations are basal and they are susceptible when progesterone concentrations are increased. The high estradiol concentrations that occur at estrus and parturition cause changes in number and proportions of circulating white blood cells, with a relative neutrophilia and a "shift to the left". Moreover, at estrus, the blood supply to the uterus is increased under the influence of estradiol, whilst at parturition there is a massive blood supply to the gravid uterus. This increased blood supply, coupled with the migration of white blood cells from the circulation to the uterine lumen, enables vigorous and active Phagocytosis of bacteria to occur. Estradiol also causes an increase in the quantity and nature of vaginal mucus, which also plays an important role in defense of the uterus against bacteria by providing a protective physical barrier and by flushing and diluting the bacterial contaminants.

**Non-specific infections/transient endometritis:**

Non-specific infections usually include normal commensal organisms that in normal circumstances do not produce disease. The most common cause of uterine infection is the pathogenic microorganisms affecting productivity and fertility of cows. Pathogenic organisms isolated from an infected uterus are found generally in livestock environments and are capable of infecting other tissues and organs. Thus, uterine infections are classified as non-specific infections. They are called nonspecific infection because the initial colonizing bacterium is not known and the specific bacteria causing the signs of infection are not known. Even though, numerous bacteria in a variety of combinations have been isolated from infected uterus, *Archanobacterium pyogenes* and *Escherichia coli* are usually associated with uterine
infection in cattle. The composition of the uterine flora changes somewhat at each recontamination, no specific combination of organisms is associated consistently with postpartum infections. Nevertheless, *A. pyogenes* either alone or in combination with other bacteria such as the anaerobic *Fusobacterium necrophorum* and *Bacteroides* spp. often is associated with uterine infections. Intra uterine oxygen reductase potential fell in the presence of infection and mostly the aerobic bacteria, thereby creating an anaerobic environment. This drop in intrauterine oxygen reductase potential may be associated with either microorganism metabolism or increased oxygen consumption by polymorphonuclear inflammatory cells. Of the anaerobic bacteria cultured from cases of uterine infection, *Fusobacterium necrophorum* and *Bacteroides* spp. have been identified. When *A. pyogenes* was isolated from uterine fluids approximately 21 days postpartum, cows developed severe endometritis and usually were infertile at first service. In addition, 69% of cows with uterine infections harbored *A. pyogenes* often in pure cultures. Others reported that 64% of the infected cows harbored *A. pyogenes* usually in combination with *E. coli*, neither study was designed to determine the prevalence of strict anaerobes. The prevalence of anaerobic and strictly anaerobic organisms, 27% of infected cows harbored *A. pyogenes* and suggested that severe uterine infections might depend on pathogenic synergism between *A. pyogenes* and anaerobic organisms such as *Fusobacterium necrophorum*. Organisms other than *A. pyogenes* and Gram-negative anaerobes such as *Fusobacterium necrophorum*, as well as, *E. coli*, *Streptococcus* spp., *Staphylococcus* spp., and *Pseudomonas* spp. are responsible for toxic puerperal metritis. Others indicated clearly that *A. pyogenes* either alone or in combination with other organisms (i.e. *Fusobacterium necrophorum*, *Bacteroides* spp., and *E. coli*), can be used consistently to induce uterine infections in cows during the puerperal or luteal phase. The growth of anaerobic bacteria may enhance the establishment of *A. pyogenes* and lead to the development of severe uterine infections. Indeed, *Fusobacterium necrophorum* produce leukotoxin, while *Bacteroides* spp. produce substances that prevents bacterial phagocytosis and *A. pyogenes* produce a growth factor for *Fusobacterium necrophorum*. *Bacteroides* and *Fusobacterium* species are prevalent in the indigenous flora on all mucosal surfaces. Tissue necrosis and poor blood supply lower the oxidation–reduction potential, thus favoring the growth of anaerobes. In addition, *Fusobacterium necrophorum* is frequently a secondary invader and mixed infection with *A. pyogenes* is common. In addition *F. necrophorum* produces a variety of extra cellular products including hemolysin, hemagglutinin, adhesions, platelet aggregation factor, proteases and DNase. The significance of these products relative to virulence is not clear. Others suggested that the earlier appearance of *E. coli* in the uterus by affecting the phenotype and function of polymorphonuclear cells, and this might support the co-infection on by *A. pyogenes* at later time.

**POSTPARTUM UTERINE INFECTION:**

Postpartum metritis is one of the most important disorders in cattle, causing high economic losses due to prolonged days open and prolonged intercalving intervals, resulting in involuntary culling. Uterine function is often compromised in cattle by bacterial contamination of the uterine lumen after parturition; pathogenic bacteria frequently persist, causing uterine disease, a key cause of infertility. The presence of pathogenic bacteria in the uterus causes inflammation, histological lesions of the endometrium, delays uterine involution and perturbs embryo survival. In addition, uterine bacterial infection, bacterial products or the associated inflammation, suppress pituitary LH secretion and perturbs postpartum ovarian follicular growth and function, which disrupts ovulation in dairy cattle. Thus, uterine disease is associated
with lower conception rate, increased intervals from calving to first service or conception, and more cattle culled for failure to conceive. Toxic puerperal metritis (i.e. acute septic metritis) is characterized by increased rectal temperature, depression, anorexia, fetid watery vulvar discharge. Toxic puerperal metritis can be a severe problem, and uterine infections that are life threatening. Metritis and endometritis are inflammation of the uterus. Metritis involves the endometrium, the underling glandular tissues and the muscular layer. While, endometritis, is involves only the endometrium with the underlying glandular tissues, without any systemic signs. These diseases share common etiological factors, predispose to one another and, largely, share common treatment.

**Treatment:**
The following criteria are important for choosing antibiotics for the treatment of uterine infections:

1. The antibiotic should be active against the main uterine pathogens and should maintain its activity in the environment of the uterus. The isolation of anaerobic bacteria from the postpartum uterus has resulted in recognition of this site as an anaerobic environment. Therefore, antibiotics that are ineffective under anaerobic conditions, such as the aminoglycosides are not recommended for the treatment of postpartum uterus. The uterine lochia consists of organic fluids and debris and contains a variety of Gram-positive and Gram-negative aerobic and anaerobic bacteria. Consequently, a broad-spectrum antibiotic that is active in the presence of organic debris is indicated. This eliminates the sulfonamides, which are ineffective in the presence of tissue breakdown products. Intrauterine administration of penicillin would also not be recommended owing to likelihood of penicillinase production by some of the bacterial species present.

2. The antibiotic should be present in a sufficient concentration at the site of infection (i.e. the sub-endothelium). This depends on the properties of both antibiotics and the vehicle. The pharmacokinetic properties of the antibiotic preparation should allow the rapid distribution of the antibiotic throughout the uterine cavity, and good penetration of the antibiotic into the endometrium.

The preparation should not inhibit the normal defense mechanisms and should be well tolerated and not induce irritation in the endometrium. Antibiotic therapy cannot sterilize the uterus nor prevent the continual recontamination that occurs during the early postpartum weeks. Successful resolution of a uterine infection requires effective uterine defense mechanisms. Cows with an abnormal puerperium, including cows with dystocia, retained placenta, or metritis have decreased activity of uterine phagocytes. In addition, most antiseptics and many antibiotics have been shown to depress phagocytosis for several days after intrauterine administration. This effect is most pronounced after intrauterine infusion owing to the high concentrations achieved in the uterine lumen. The usage of lugol’s iodine solution destroyed phagocytic activity of the uterine leukocytes for several days after intrauterine application.
Examination of CervicoVaginal Mucus Sample

Examination of cervico-vaginal mucus sample help in the assessment of physio-pathological condition of female genital organs.

1. Colour
   - Transparent - Normal (in oestrus period).
   - Scanty reddish colour discharge - Metoestrus phase
   - Opaque or transparent with flakes - Mild infection
   - White or yellow colour - Metritis/ pyometra

2. Consistency
   - Thin watery - Early oestrus
   - Viscous and ropy - Mid heat
   - Thick - Late heat

3. Odour - Normally, genital discharge is odourless. However, foul smelling odour generally indicates severe metritis with systemic involvement with possible retention of foetal membranes or some foetal parts in the uterus. It is usually found in cases of post-parturient disorder.

4. pH : The pH of genital discharge can be recorded by an ordinary pH indicator paper or using pH meter. The normal pH of genital discharge ranges from 6.5 to 7.4. A higher pH indicates presence of infection.

5. White side test:
   - Take 1 ml. cervical mucus in a sterilized test tube.
   - Add 1 ml 5% sodium hydroxide solution to it.
   - Heat the mixture upto its boiling point.

Interpretation :
   - Dark yellow colour - Clinical metritis
   - Yellow colour - Subclinical metritis
   - No colour - Normal

6. Microbiological examination: The discharge is sent for isolation and identification of organisms and for antibiotic sensitivity test.

Pregnancy Diagnosis for cows

EXTERNAL EXAMINATION:

(a) Visual examination:
   - Cessation of oestrous cycle after artificial/ natural insemination.
   - Sluggish and docile behaviour.
   - Fattening tendency particularly during early pregnancy.
   - Gradual drop in milk yield (after 5 months)
   - Gradual increase in body weight.
   - Gradual increase in the size of the abdomen.
   - Flanks become hollow and spine appears more prominent.
   - The size of mammary glands/ udder begins to increase from about 5th months of gestation in heifers, while in older cows it is usually observed just 2-3 weeks before parturition.
   - In few animals, a prepartum udder oedema and umbilical oedema is noticed.
(b) Abdominal ballotment :
- Abdominal ballotment of foetus on the right side of the animal can be done from ~ month onwards (Fig. 11.1).
- Press abdomen (Rt. side) by closed fist and release suddenly and apply the palm against the abdominal wall to feel the foetus which hits the palm.
- A 7th month foetus is felt very near to the ribs and 9th month foetus is felt near the udder.
- Therefore, abdominal ballotment should be performed at proper site as mentioned above.

Fig. 11.1 : Rough estimation of the month of pregnancy by means of deep palpation of the flank, using clenched fists

(c) Drenching cold water :
- Drenching cold water causes the foetal movement from ~ month onwards.
- In the early morning, the animal is faced towards north and cold water is drenched or the animal is allowed to drink cold water.
- Examiner should stand near the head of the animal.
- When sun-rays fall on the abdomen, the foetal movement can be well-appreciated in pregnant animal.

RECTAL EXAMINATION (see Fig. 11.2, 11.3, 11.4, 11.5 & 11.6) :

First month (Negative stage) -
- Both the uterine horns are symmetrical.
- Uterine horns are intrapelvic.
- Feel of uterine horn is normal.
- One of the ovaries exhibits CL.
- Cervix remains closed.

Second month (31st to 60th days) or small sac stage:
- Uterus is usually intra-pelvic and palpable from all the sides. • Uterus is tonic.

Fig. 1 : Rectal examination of the female genital system in the 70th day of pregnancy.
Fig. 2: Rectal examination of the female genital system in the 90th day of pregnancy.

Fig. 3: Rectal examination of the female genital system in the 110th day of pregnancy.

- Pregnant horn is 2-4 times enlarged.
- Slippery feel of foetal membrane when horn is palpated between fingers (double wall) from the 5th week of pregnancy in heifers and from the 6th week in cows (placental palpation).
- Uterine wall thinner than normal due to increased diameter of uterine horn.
- Ovaries are at normal position and one of the ovary exhibits pregnancy CL or corpus luteum verum, which differs from periodic corpus luteum in not having a neck.
- Cervix is closed and normal in position.

Corpus luteum verum is slightly longer in diameter (2.5 cm) and weight (6.5 gm) than the CL of oestrus cycle (CL spurium) which is 2.3 cm in diameter and 5.7 gm in weight.

Third month (61st - 90th days) or large sac stage:
- Now, uterus hangs on the brim of pelvis and is palpable from only three sides.
- Uterus is tonic
- Pregnant horn is further enlarged.
- Thinning of uterine wall continues (very thin).
- Rebound effect is detectable.
- Ovaries are pulled forward.
- Cervix is stretched, pulled forward.
- Heaviness is felt when cervix is bulled by examiner.

Fourth month (91st - 120th days) or Balloon stage:
- Uterus is abdominal.
- Thinning of uterine wall continues.
- Cotyledons are detectable.
- Fluctuations can be felt.
- Fremitus (+) can be felt.
- Cervix is located beyond/ at pelvic brim (reason due to increase in weight of uterus, so it is pulled forward).
- Ovaries are pulled forward and are out of reach i.e. in abdominal cavity.

Fifth month (121st - 150th days) or sinking stage:
- Uterus is sinking in abdomen.
- Foetus and fluctuations are felt.
- Cotyledons are bigger in size (3.5 cm)
• Fremitus (++) can easily be felt.
Sixth to seventh month (15pt - 210th days):
• Uterus is entirely abdominal.
• Foetus sinks more deep in the abdominal cavity and is not palpable.
• But in the last of seventh month, foetus starts to come near the pelvic cavity and is easily palpable.
• Fremitus (+++) is strong.
• Pregnancy diagnosis is easy in heifers than cows.
• Early pregnancy diagnosis (35th - 45th days) by inexperienced clinician may results into abortion.
Reason: Excess pressure applied during manipulation of the vesicles and embryo results rupture of amniotic vesicles and embryonic death. The most common cause of embryonic death is rupture of the heart or the vessels at the base of the heart resulting in hemorrhage into amniotic cavity.
Eight to ninth month (21pt - 270th days):
• Foetus comes again nearer to the pelvic cavity.
• Foetal parts can be clearly felt.
• Fremitus (++++) is very strong.
• Size of the cotyledons increases to about tennis ball size (7 - Bcm.).
• Foetal bumps are felt when foetus is pressed in the abdominal cavity.
• Strong foetal movement is palpable.
Slipping of foetal membrane:
- Early pregnancy diagnosis (from 35 to 90 days) can be best performed by palpating foetal membrane. However slipping of foetal membrane occurs throughout gestation from 35 days but in late stage of gestation other things are more important than slipping of fetal membranes.
- The technique consists of gently picking up and pinching or compressing pregnant horns of the uterus and feeling the foetal membranes (chorio-allantois) which slip between the thumb and the fingers before the uterine wall escaped from between the fingers.
- This technique of slipping the membranes is especially valuable in the differential diagnosis of pregnancy from uterine disease such as pyometra and mucometra.
- It is important to note that the entire diameter of each uterine horn must be palpated so that if foetal membrane slip is present, it will not be missed.

Palpation of amniotic vesicle:
- From approximately 30 to 65 days of gestation, the amniotic vesicle can be detected as a movable oval object within the uterine lumen.
- The vesicle is rigid in early pregnancy but becomes flaccid with advancing gestation (after 65 to 70 days) when it is difficult to detect at all. Palpation of placentomes:
  - The presence of placentomes is another positive sign of pregnancy.
  - These are palpable from about 75 days to term.
  - These are palpated as soft, thickened lumps in the uterine wall and more easily detected as pregnancy advances.
  - In general, the placentomes in the middle of the gravid horn and nearest the attachment of the middle uterine artery are larger than those placentomes in the cervical or apical end of the horn or in the opposite horn.

Palpation of foetus:
- The foetus can be palpated from the time of amniotic softening (65 to 70 days) to term.
- Palpation of foetus before 60 to 70 days of gestation is not possible because of the tense and distended amniotic vesicle, and the small size of the foetus.
- After 60 to 70 days in heifer and small breeds of cow, foetus can be palpated throughout gestation period.
• In heavy breeds of cow, foetus may not be palpable in mid-gestation. In that condition, pregnancy diagnosis is based on the position of the uterus, the size of uterine artery, palpation of placentomes and slipping of foetal membrane.
  • After the sixth month of pregnancy, foetal movement can be stimulated by pinching the claws of foetus, grasping and pulling of foetal leg, pinching the eye balls or grasping the nose of the foetus through rectal wall.
  Palpation of fremitus:
  • The major blood supply to the gravid uterus is the middle uterine artery; which gets enlarged considerably as pregnancy progresses.
  • The blood supply to the left horn is by left middle uterine artery and the blood supply to the right horn is by right middle uterine artery.
  
Origin:
• The uterine artery is originated from the pudendal artery at the level of the iliac crest and travel in the broad ligaments.
• Because of their location in the broad ligaments, they are freely moveable and can be differentiated from the external iliac arteries which are tightly attached to the medial shaft of each ilium.
• Thus, one should not confuse with the external iliac artery (it also passes through shaft of ilium) because it does not move when pulled, while middle uterine artery moves when pulled.
  
Technique of palpation:
• The right middle uterine artery can be palpated by directly applying fingers over the right lateral wall of the pelvic cavity, and it is felt while the left middle uterine artery is examined by rotating the hand in a clockwise direction and applying finger over the left lateral wall of the pelvic cavity and its inlet.
• In pregnant animal, a sensation is felt like when a person presses a rubber pipe for partial obstruction of water flow. This sensation or turbulence created by blood flow is called fremitus or whiring or thrill.
• In early pregnancy, it may be necessary to place very slight pressure on the artery to elicit the fremitus, but as pregnancy progresses the buzzing becomes obvious without pressure.
  
Importance:
• Fremitus gives idea about the horn containing foetus.
• Enlargement of ipsilateral middle uterine artery to the pregnant horn is detectable after 90 days of gestation.
• By approximately 120 days, the blood flow within the ipsilateral middle uterine artery has increased to the point at which turbulence is palpable as a buzzing sensation, also referred to as a thrill or fremitus.
• The middle uterine artery which supplies blood to the non-pregnant horn, also increases in thickness but the change is slower than the artery of pregnant horn.
• By about 7 to 8 months, the fremitus is often palpable in the contralateral uterine artery.
• The presence of bilateral fremitus before 7 to 8 months, especially when the two arteries are symmetrical, this feature strongly suggests bicornual twins.
• If the fremitus was felt in earlier pregnancy and then disappears indicates death of the fetus.

*Due to increase in the diameter of middle uterine artery the arterial wall becomes thinner as pregnancy advances. Blood flow also increases as pregnancy advances.*
This is why, instead of feeling a pulsation in the artery (normally) a characteristic ‘fremitus’ is felt.

- The uterus of heifers is usually located in the pelvic cavity until 3 to 4 months of pregnancy.
- In all ages of cattle, the uterus lies on the floor of the abdominal cavity after the 4th month of pregnancy.
- From 5th to 6th month of pregnancy, the uterus sinks deeply in the abdomen so that in the larger breeds, only cervix and middle uterine artery (fremitus) can be palpated per rectum.
- From 6th to 7th month, the foetus becomes large enough so that it can be again palpated on rectal examination in almost all cows.
- From 8th to 9th month, the foetus may extend caudally so that nose and feet are resting in the pelvic cavity.

**Early Pregnancy Diagnosis in Cows by Milk Ejection Test**

**OBJECTIVES:**
- To confirm the pregnancy after 20-22 days of insemination.
- To reduce duration of service period.

**PRINCIPLE:**
PGF₂ alpha luteolytic dose induces the release of oxytocin from the corpus luteum which causes let-down of milk in the lactating and pregnant cows.

**PROCEDURE:**
- This test is performed generally 12-18 hours after insemination.
- Place the teat cannula in the left fore-teat and leave it for milk flow from teat cistern.
- When the milk flow ceases, a small dose (2.5 mg or 0.5 ml) of Dinoprost (Lutalyse) is administered intravenously through ear vein.
- If the corpus luteum of pregnancy is present, alveolar milk starts to flow about one to two minutes later.

**Differential Diagnosis of Pregnancy in Bovine**

**OBJECTIVE**
To prevent false positive diagnosis. There are many cases in which uterus remain distended and an inexperienced person may make false positive diagnosis.

**Pyometra:**
- Pus remains present in the uterus.
- Both the uterine horns remain equally distended while in pregnancy, horns remain asymmetrical.
- Fremitus is absent in pyometra because there is no need to supply extra blood.
• Thick uterine wall and lack of tone while in pregnancy, thin and tonic uterine wall.
• No dorsal bulging of the horn like as in case of pregnancy because pus tends to gravitate and collect in dependent portions of the horns.
• Placentomes are absent.
• No slipping of foetal membrane.
• If the diagnosis is uncertain, re-examine after one or two months. In normal pregnancy, progressive development of the foetus and uterus occurs, whereas in pyometra, the condition remains essentially same.

Mucometra or hydrometra:
• No slipping of foetal membrane
• No placentomes
• No fremitus
• Failure of progressive development of uterus as in a normal pregnancy.

Mummification:
• Solid mass tightly surrounded by uterine wall.
• No placental fluids.
• Intra-abdominal uterus may be confused with pregnancy.
• No increase in the size of abdomen.
• No placentomes
• No fremitus.
• If ovary is palpable, CL is present just like as in pregnancy.

Foetal maceration:
• No fremitus
• No placentomes
• Cervix is partially closed
• Skeletal part of foetus keeps on floating within lumen of uterus which gives a characteristics crepitating feeling or gritty feeling.
• Apart from these conditions, an experienced person may confuse with visceral organs of the animal.

Ovaries - may be confused with cotyledons.
Distended ventral sac of rumen - may be confused with foetus.
Distended urinary bladder - may be confused with pregnant horns.
• Anatomically, there is no reason for confusing a pregnant uterus with such structures. Careful rectal examination, consideration of the anatomical structures and relationships of these organs and their consistency will prevent erroneous diagnosis.

Biological and Chemical Methods of Pregnancy Diagnosis

Biological methods:
1. Ascheim Zondek (A-Z) Test: This is a biological test utilized for diagnosis of pregnancy in mare. This test is based on FSH like activity of PMSG, present in the blood of pregnant mare. This test is more accurate between days 50-100 post conception.
Procedure:
• Collect 30-40 ml. mare's blood from the jugular vein.
• Allow it to coagulate for 24 hours and separate the serum.
• Store the serum at 4°C.
Inject 0.25 ml serum subcutaneously twice daily for 2-4 days to an immature female rat of about 22 days of age.

Kill the rat % or 120 hours later and inspect the genital organ.

Interpretation:
If mare is pregnant the genitalia of rat will have following changes.
- Ovaries: Many haemorrhagic spots or corpora haemorrhagica appear as dark-red or black spots.
- Uterus: Oedematous.
2 to 4 times of normal size.
A little amount of fluid remains present in the uterus.
- Vagina and Vulva: Swollen.
Vaginal swabs show many cornified epithelial cells.

If mare is not pregnant:
No definite change in ovaries and uterus of rat.

2. Friedman test or Rabbit test:
- It is not commonly performed because of the cost of rabbit.
The basic procedures in this test is similar to rat test but the age of rabbit, the dose of the serum injected will vary.
- 2 ml of mare's serum is injected intravenously in the ear vein of immature female rabbit {19 to 20 weeks old}.
- Perform laparotomy after 24 hrs. of injection.
- Presence of corpora haemorrhagica in the ovaries and oedematous condition of the uterus indicate positive diagnosis.

Chemical methods:
(i) Cuboni Test:
- This test is used for pregnancy diagnosis in mare.
- This test involves detection of oestrogen in the urine of mare and can be performed after 150 days of conception
Principle - The urine is hydrolysed with HCl, and benzene is added for extraction of oestrogen from hydrolysed urine.
Method -
- Take 15 ml urine in a test tube and add to it 3 ml cone. HCl.
- Heat the mixture in a water bath at boiling point for 10 minutes.
- Cool the mixture.
- Pour the mixture into a separating funnel and add to it 18 ml benzol and shake well.
- Collect the benzol layer in an other test tube and add to it 10 ml cone. H:zSOa.
- Heat the mixture at 80°C for 5 minutes and cool.
Interpretation:
- Green fluorescence
- No colour

(ii) Barium chloride test:
pregnant
Non-pregnant
- This test is used for pregnancy diagnosis in cattle and buffalo.
- It gives more than 90% reliable results.
Principle - End-products of progesterone (after metabolisation in liver) present in the urine and this prevents precipitation of barium chloride while oestrogens favour precipitation.

Method:
- Take 5 ml of urine in a test tube.
- Add 5-6 drops of 1% barium chloride solution and mix well.

Interpretation:
- Clear white precipitation: Non-pregnant
- No precipitation: Pregnant

Advantage: Pregnancy can be diagnosed even at 3 to 4 weeks of gestation.

Limitation:
- When oestrogens in urine are of plant origin, it may give wrong result.
- Presence of persistent corpus luteum and corpus luteum of pregnancy up to some days after parturition give false positive result.

(iii) Sodium hydroxide test:
- This test is used for pregnancy diagnosis in cattle and buffalo.
- This test has a reliability of 80-90%.

Method:
- Take 0.25 ml of cervical mucus in a test tube.
- Add to it 5 ml 10% solution of NaOH.
- Heat it till boiling.

Interpretation:
- Orange: Pregnant
- Pale colour: Non-pregnant

(iv) Specific gravity method: This test has more than 90% reliability both in cows and buffaloes.

Principle - Specific gravity of cervical mucus is increased with progesterone while it is decreased with oestrogens.

Method:
- Take few ml. of copper sulphate solution having specific gravity 1.008 in a test tube.
- Add 0.25 ml cervical mucus in the copper sulphate solution.

Interpretation:
- If mucus sinks: Pregnant
- If mucus floats: Non-pregnant

Seed bio-assay method:
- This method is used for pregnancy diagnosis both in cows and buffaloes.

Principle - Germination of wheat/barley is prevented by four-fold rise in concentration of abscisic acid in the pregnant animals. It induces dormancy in seeds.

Method:
- The urine is collected and diluted four times with distilled water.
- Two petri-dishes are taken and filter papers are placed in it.
- About 15-20 wheat seeds are kept in each petri-dish.
- About 10-15 ml of the above diluted urine sample is added to one petri-dish, while in other petridish only water is added (control).
• Cover the petri-dishes to prevent evaporation and keep for 5 days.

Interpretation:
• No germination and turn black or if germinate but shoots are less than 1cm. in length - Pregnant.
• 35-60% germination with moderate shoot length (4 cm.) - Nonpregnant.
• Control petri-dish - 60-80% germination and shoot length about 6 cm.
Gynaecological Examination of Vagina:

OBJECTIVE:

To know the physiological and pathological condition of vagina.

MATERIALS REQUIRED: Vaginal speculum, liquid paraffin, soap, water etc.

PROCEDURE:

- Restrain the cow in a crate or trevis.
- Clean the vulva and adjacent parts with cotton dipped in normal saline or antiseptic solution.
- Lubricate the sterilized vaginal speculum with liquid paraffin or soap water.
- Insert the speculum through the vulva into vagina while keeping the jaws of speculum closed to avoid injury.
- Turn the handle of vaginal speculum either downward or upward and open the jaws.
- Use torch to observe the anterior part of vagina and outer part of cervix.
- Note the finding like discharge, vaginitis, abscess, tumour of cervix (open or closed), cervicitis etc.
- Remove the speculum in an open fashion.

INTERESTING FACTS

- Speculum examination is totaly contra-indicated in pregnant animals and in animals suffering from severe vaginitis and other painful conditions.
- Vaginal examination, AI or intrauterine treatment is contraindicated when the vulvar lips are wet or soiled.

Technique of Intra-uterine Therapy

OBJECTIVE:

To introduce the drugs into the uterus to overcome the infection in various disease conditions.

MATERIALS REQUIRED:

Obel's apparatus, catheter, syringe, cotton, saline, pipettes, etc.

PROCEDURE:

- Clean vulva and perineal region with the dry cotton.
- Insert the left hand in the rectum and remove the faecal material by back racking.
- Spread vulva apart and insert the instrument (catheter or Obel's apparatus) up to fornix.
- Hold the cervix between two fingers through rectal wall and keep thumb on the external os.
• The catheter is initially inserted pointing upwards at an angle of about 300 to avoid entering into the external urethral opening and is then moved horizontally until it is engaged in the external os of the cervix.

• Entry into the external os is accompanied by a characteristic 'gritty' sensation.

• Thereafter, introduce the catheter through convoluted cervical canal by manipulation of the cervix through rectal wall.

• Place one finger over the internal os of the cervix, so that the tip of the catheter can be palpated when it passes the cervical canal.

• As soon as, the catheter is passed, the drug should be pushed through syringe into the body of uterus not in uterine horn.

• In this way, drug is equally distributed between the two uterine horns.

Important Facts
The recto-vaginal method of intrauterine medication requires considerable practice for success.

• Obstruction in passing catheter by vaginal folds can be minimized by pushing the cervix forward. By doing this, vaginal passage becomes unfolded.

• After passing catheter in the cervix, no forward pressure should be exerted on the catheter with the right hand because uterine wall is friable and easily penetrated if the catheter moves suddenly.

• The most common fault during intra uterine therapy is twisting of cervix in the left hand which occludes uterine horns.

Interesting Facts
The Irritant solutions such as Logol's iodine or tetracycline's when given intrauterine affect length of estrous cycle.
The irritant intrauterine infusions given during days 3 to 9 of the cycle (oestrus day 0) may significantly shorten the time for the female to return in oestrus.
The infusions at oestrous or mid-diestrus do not affect oestrous cycle length.
The infusions on days 14 to 17 of the cycle (oestrus day 0) prolong the luteal period or estrous cycle length.

Collection of Genital Discharge
OBJECTIVE:
To examine the genital discharge to have an idea about different types of infections, severity of condition, diagnosis and its treatment.
MATERIALS REQUIRED:
Catheter, pipette, cotton gauze, syringe and sterilized bottle.
METHODS:
A. By back racking -
• After inserting the hand into rectum, slightly lift the uterus and cervix upward and massage in backward direction.
• Thereafter, massage backwardly to the vagina through rectal wall which will result in flow of cervical mucus through the vulvar lips.
• Collect the discharge in a wide-mouthed sterilized test tube.
• Cut the discharge with the help of scissors when it remains hanging.
B. By catheter/pipette:
• Introduce the catheter into vagina (anterior part) or cervix or from where genital discharge has to be collected.
• Suck the mucus with the help of syringe which remains attached to the other end of catheter or pipette.
C. Tampon method:
• Take a sterile gauze tampon of about 1 g. and attach a string to it.
• Insert the sterilized gauze tampon into the vagina.
• Leave the tampon in the vagina for 20 min.
• Remove the tampon from the vagina by pulling the string.
• Place it in a sterilized bottle containing saline.