LECTURE 3

Reproductive Physiology and Endocrinology

A. Reproductive endocrinology

1. Reproductive organs and hormones of the endocrine system;
Endocrine control of estrus cycles, pregnancy, spermatogenesis, sexual development and behavior and all other aspects of reproduction is the responsibility of the **hypothalamic-pituitary-gonadal axis**. Hypothalamic hormones control the release of pituitary hormones while pituitary hormones exert their effects on the development, function and hormone synthesis of the gonads.

Gonadal hormones, in addition to direct effects on the genital organs, complete the hypothalamic-pituitary-gonadal axis by either suppressing or stimulating the production and/or release of hypothalamic and pituitary hormones in what is called negative feedback or positive feedback mechanisms. Gonadal Hormones also exert influence over growth, behavior, organs or tissue function such as in the mammary glands. Most other endocrine systems have similar feedback mechanisms.

A) **Hypothalamus**; the master endocrine gland;
The hypothalamus represents the interface of the central nervous system with the endocrine system. The hypothalamus receives direct CNS innervation from several areas of the brain stem and cerebrum. Direct and indirect visual, olfactory, auditory and touch sensory inputs also innervate areas of the hypothalamus. Neurons of the hypothalamus involved with reproduction also contain receptors for estrogen and other steroidal hormones, constituting the feedback mechanism. These neuronal and hormonal signals regulate the endocrine functions of the hypothalamus.

Anatomically the hypothalamus is a diffuse area of the brain, composed of groups of neurons organized into distinct nuclei. These nuclei lie dorsal and rostral of the pituitary gland and stalk, in an area surrounding the third ventricle, in the most rostral portions of the brain stem.

Functionally there are two primary divisions of the hypothalamus:

1) Direct innervation of the **posterior pituitary gland**; the main hypothalamic neurons innervating the posterior pituitary originate in the paraventricular nucleus and the supraoptic nucleus. These neurons release the posterior pituitary hormones **oxytocin** and **vasopressin**. This portion of the hypothalamus will be discussed below under the posterior pituitary discussion.

2) Specialized hypothalamic neurons that secrete **Releasing Hormones** into the **Hypothalamic-Pituitary Portal Circulation System**. These releasing hormones control the function of the **anterior pituitary gland**.

a. Individual releasing hormones target specific anterior pituitary gland secretory cells and stimulate the synthesis and release of a specific anterior pituitary hormone.

b. The neurons that secrete **Gonadotropin Releasing Factor (GnRH)**, for instance, originate in various nuclei in the mediobasal area, preoptic area and mamillary body of the hypothalamus. The axons from these neurons (and neurons producing the other releasing hormones) end on the capillaries of the pituitary portal system in the median eminence just anterior and dorsal to the pituitary stalk.

c. GnRH is released from the axons, enters the portal circulation and is carried to the anterior pituitary. In the anterior pituitary GnRH is bound to specific receptors on pituitary gonadotrope cells and elicits the release of follicle stimulating hormone (FSH) and Luteinizing hormone (LH).

d. Other hypothalamic releasing hormones;

1. **Thyrotropin Releasing Hormone (TRH),**
2. **Corticotropin Releasing Hormone (CRH),**
3. **Growth Hormone Releasing Hormone (GHRH)**
4. Somatostatin

B) **Pituitary Gland**;

The pituitary gland lies at the base of the brain stem just posterior to the optic chiasm. It is a ventral protuberance composed of a stalk or **infundibulum**, the **anterior lobe** or **adenohypophysis**, the **posterior lobe** or **neurohypophysis** and an intermediate lobe that is part of the adenohypophysis.

1) **Posterior Pituitary gland** or neurohypophysis is composed of axons and nerve endings from neurons originating in the supraoptic and paraventricular nuclei of the hypothalamus. The cell bodies of these neurons are in the hypothalamus while the axons terminate in the posterior pituitary gland and are the site of hormone release. The neurohypophysis is functionally part of the hypothalamus and thus a direct extension of the CNS.

Posterior pituitary hormones are released from these neurons as part of specific physiological reflexes. The hormones, **Oxytocin** and **Vasopressin**, are small peptides (9 amino acids) which have very similar structure. These hormones are analogous to neurotransmitters but have their effect at a distant organ.

a. Oxytocin; causes smooth muscle contraction

1. Released as part of the suckle reflex, causes contraction of the myoepithelial cells of the alveoli, resulting in milk let-down
2. Also involved in uterine contraction at parturition and immediately post-partum.
3. Elevated estrogen levels are required for induction of oxytocin receptors in the myometrial cells of the uterus

a) Injected oxytocin will cause uterine contraction at the time of parturition (estrogen levels are high)

b) By about two days postpartum, oxytocin will not cause uterine contractions

b. Vasopressin (or antidiuretic hormone)

1. Renal resorption of water “dehydration reflex”
2. Vaso-constriction and blood pressure

2) **Anterior Pituitary gland** or adenohypophysis is composed of several cell types, each of which secretes a different tropic hormone under the control of hypothalamic releasing hormones. The cell type include:

a. Gonadotrope; producing **FSH** and **LH** when stimulated by GnRH

b. Lactotropes; producing **Prolactin**, release inhibition by dopamine

c. Somatotropes; producing Growth Hormone (**GH**) when stimulated by GHRH

d. Corticotropes; producing Adrenocorticotropic Hormone (**ACTH**, also called corticotropin) when stimulated by CRH

e. Thyrotropes; producing Thyroid Stimulating Hormone (**TSH**, also called thyrotropin), when stimulated by TRH

All of the anterior pituitary hormones are very large polypeptides (GH and Prolactin) or complex glycoprotein (FSH, LH and TSH) except ACTH, which is a relatively small 39 amino acid polypeptide.

FSH and **LH** are dipeptide glycoproteins that contain 2 polypeptide subunits, which are glycosylated (contain attached sugar molecules), and are secreted from gonadotrope cells under GnRH stimulation.

FSH

a. FSH target cells

1. Sertoli cells
2. Granulosa cells

b. FSH main functions

1. Stimulates follicular growth
2. Stimulates spermatogenesis
3. Aromatization of androgens to estrogen

c. FSH Increases secretion of paracrine peptides at the level of the ovary and testes, which increase cell proliferation.

LH

d. LH target cell

1. Leydig cells
2. Thecal cells
3. Granulosa cells pre-ovulatory only

e. LH main functions

a. Production of androgens and progesterone

b. Induction of ovulation

Prolactin is secreted by the lactotrope cells of the anterior pituitary gland. It is a large single chain peptide hormone

a. Prolactin target cells
   1. Mammary gland secretory cells
   2. Follicular and seminiferous tubule cells
b. Prolactin primary functions
   1. Stimulate mammary gland development
   2. Induction and maintenance of lactation
   3. Induction of maternal behavior
   4. Stimulates immune response; particularly the formation of colostral antibodies
c. High levels of prolactin have a profound modulating effect on reproduction. These effects have a negative impact on reproduction efficiency and are responsible for lactational and seasonal anestrus and anovulation.
   1. Antagonist to nearly all actions of FSH and LH
   2. Inhibition of testosterone and estradiol steroidogenesis
   3. Inhibition of follicle growth
   4. Inhibition of ovulation
d. Static levels of prolactin are required for some reproductive effects:
   1. Required for induction of LH receptors
   2. Helps maintain corpus luteum (CL) function and is required for progesterone production in the CL
   3. Supports testicular function including sperm cell transport in the epididymis and vas deferens
   4. Regulates accessory genital gland secretions in the male
e. Dopamine from the hypothalamus inhibits prolactin secretion and is the main regulator of prolactin secretion. For instance static levels of dopamine inhibit prolactin secretion. When offspring suckle there is a temporary decrease in dopamine in the portal circulation and the level of prolactin release surges.
f. Prolactin releasing factor has been recently discovered.

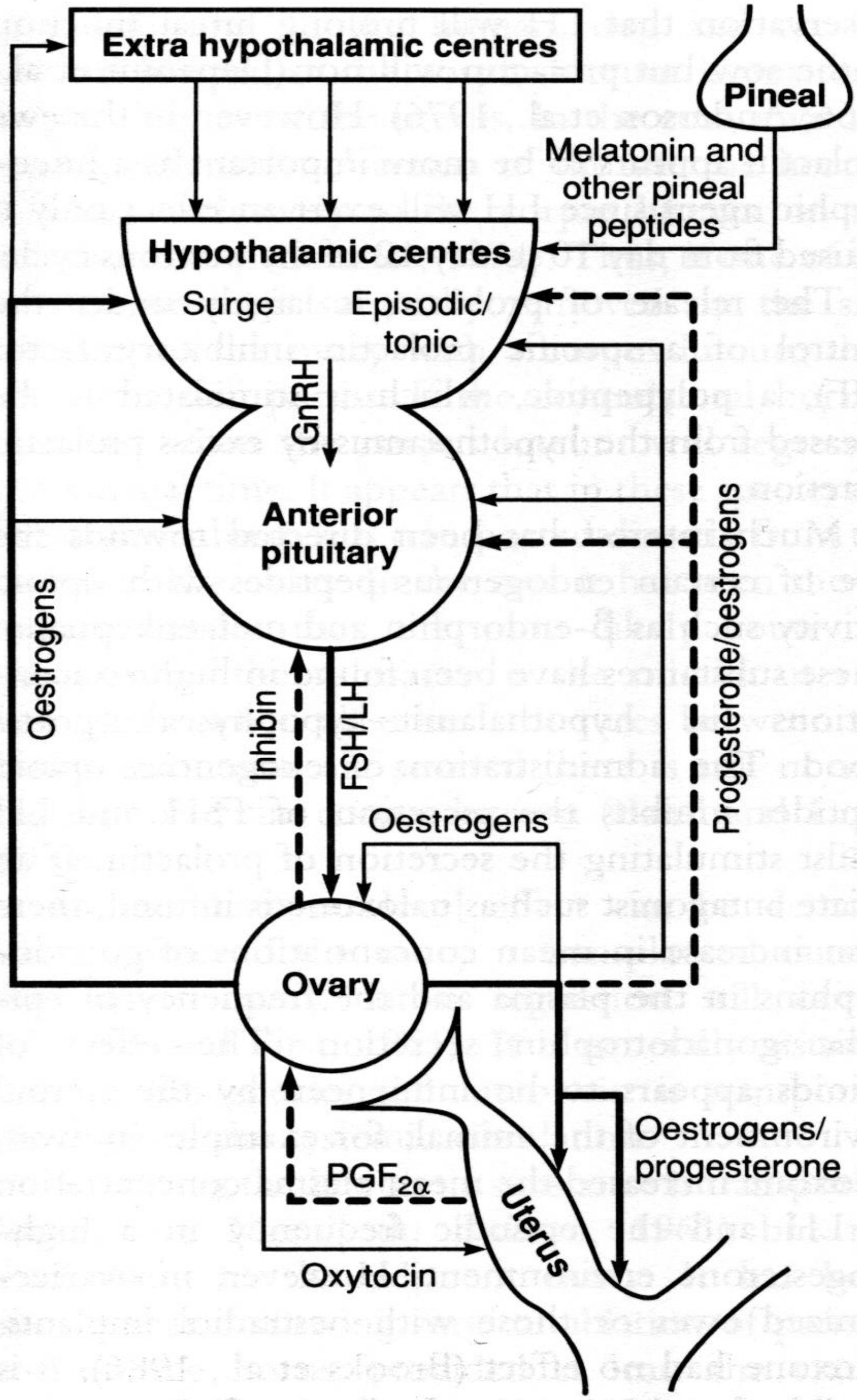

Fig. 1.1 Endocrine control of cyclical reproductive activity; ——, stimulation; - - -, inhibition; PGF$_{2\alpha}$, prostaglandin$_2$. (From Lammin et al., 1979.)

C) **Gonads**;

Spermatogenesis, oogenesis, folliculogenesis and steroidal hormone production by the gonads are all under direct positive influence of FSH, LH and the chorionic gonadotropins.

1) LH is responsible for stimulating the production of progesterone from cholesterol in thecal and granulosa cells of the ovary and in Leydig cells in the testicle.
2) LH also stimulates the conversion of progesterone to testosterone in thecal cells and Leydig cells.
3) Without LH, testosterone production would cease.
4) FSH is responsible for cellular proliferation in spermatogenesis and folliculogenesis.
5) FSH is necessary for the conversion of testosterone to estradiol in granulosa cells of the ovary and Sertoli cells in the testicle. LH is only indirectly responsible for estradiol production in that testosterone is required as a precursor for estrogen production.
6) Steroidal hormones, for the most part, have negative feedback influence on GnRH production and secretion by the hypothalamus and gonadotropin release from the pituitary gland. Castrated animals and menopausal women have very low steroidal hormone levels and a concomitant drastic elevation in FSH and LH levels. In these situations elevated gonadotropin levels result from the release of the hypothalamus from the negative feedback of gonadal hormones. Injection of steroidal hormones at doses sufficient to restore near normal circulating levels will cause a decrease in FSH and LH to pre-castration levels
7) The primary steroidal hormones produced by the gonads follow:
    a. **Cholesterol** is the precursor of steroidal hormones the first active steroidal hormone produced from cholesterol is progesterone.
    b. Pregnenolone is an intermediate precursor of progesterone
    c. **Progesterone**;
        1. Produced by:
            a. Leydig cells,
            b. Thecal cells (pre and post-ovulatory),
            c. Luteal cells derived from thecal cells
            d. Luteal cells derived from granulosa cells (post-ovulatory only),
            e. Fetal placenta
            f. Adrenal Glands (precursor for cortisol)
        2. Target cells
            a. Endometrial epithelium and glandular epithelium
            b. Mammary gland epithelial cells
            c. Hypothalamic GnRH secretory cells
            d. Lymphocytes during pregnancy
        3. Functions include:
            a. Intermediate precursor of androgens
            b. Creation of a uterine environment suitable for sustaining the developing embryo and fetus
            c. Implantation and maintenance of pregnancy

d. Inhibition of FSH and LH secretion

d. **Androgens**;

1. **Androstenedione**, precursor of testosterone and estrogen
2. **Testosterone** is produced by:
    a. Theca interna cells
    b. Leydig cells
3. Target cells
    a. Testicular and epididymal epithelium
    b. All male primary and secondary sex organs, especially during fetal development
    c. Sertoli cells
    d. Granulosa cells
    e. Hypothalamic GnRH secretory cells
    f. Neurons in other areas of the brain involved with sexual behavior (limbic system)
4. Functions:
    a. Sexual differentiation of the male genital system (a castrated male fetus will develop female genitalia and testosterone supplementation of a castrated male fetus will causes male genitalia to develop)
    b. Development of secondary sex characteristics
    c. Some functions in support of spermatogenesis
    d. Sexual and other behavior
    e. Intermediate precursor of estrogen
    f. Inhibition of FSH and LH secretion

e. **Estrogen**

1. Testosterone is the precursor of estrogen
2. Estrogen is produced by aromatization of the first ring of the testosterone molecule.
3. Produced by
    a. Sertoli cells
    b. Granulosa cells
4. Target cells
    a. Endometrial epithelium and myometrial cells
    b. Fimbria and Oviduct epithelium and muscle cells
    c. Cervical and vaginal epithelium
    c. Mammary gland secretory cells
    d. Hypothalamic GnRH secretory cells
    f. Neurons in areas of the brain involved with sexual behavior (limbic system)
5. Functions:
    a. Secondary sex characteristics
    b. Estrus behavior (in coordination with testosterone)
    c. Oocyte maturation
    d. Erection of the fimbria prior to ovulation

d. Maintenance of a suitable environment in the oviduct for fertilization and early embryo support

d. Inhibition or stimulation of FSH and LH release through modulation of GnRH secretion

1. Estrogen, at low to moderate levels, exerts negative feedback on GnRH production and thus decreases LH and FSH release by the pituitary. This action occurs via down regulation of the GnRH secreting neurons in the hypothalamus.

2. At moderate to high circulating levels, only when progesterone levels are low (less than 1 ng/ml) such as during estrus, estrogen has a positive feedback on LH release from the pituitary. High estrogen levels at estrus produce positive feedback on GnRH neurons and anterior pituitary gonadotrope cells, causing release of LH and initiating the **pre-ovulatory LH surge**.

f. The ovary and testicle also produce **inhibin** and **activin**, which have inhibitory and stimulatory influence respectively on FSH production by the pituitary.

Inhibin

1. Produced by:
   a. Granulosa cells
   b. Sertoli cells
   c. Leydig cells (to a lesser degree)
   d. Thecal cells??
2. Target cells: Pituitary gonadotrope cells are the only known cells with receptors for inhibin
3. Functions:
   a. Inhibits pituitary FSH production,
   b. Part of the mechanism by which a single follicle exhibits dominance in monotocous females

Activin is structurally related to inhibin

1. Produced by:
   a. Granulosa cells
   b. Other cells? Possibly the same cells as inhibin
2. Target cells: Pituitary gonadotrope cells are the only known cells with receptors for activin
3. Functions: The actions of activin oppose inhibin
   a. Stimulate pituitary FSH production,
   b. It is unclear what role activin has in folliculogenesis.

D) Placenta; producing steroidal and gonadotropic hormones.

E) **Pineal Gland**; Produces **Melatonin** and other monoamine hormones or neurotransmitters such as serotonin. Melatonin secretion is stimulated by dark and decreased daylight in the fall and winter. Changing melatonin levels influence the secretion of GnRH in the hypothalamus and is responsible for the initiation and termination of the breeding season in seasonally polyestrous species. Sheep, deer and other fall breeders initiate estrus cyclicity in response to increasing melatonin levels. Horses, a spring breeder, respond to decreasing melatonin levels.

F) Uterus; producing **prostaglandin F2 alpha** (**PGF2 alpha**) during the late luteal phase of the estrus cycle.

1) Prostaglandin F2 alpha is produced by endometrial cells
2) Secretion stimulated by oxytocin and estrogen
3) Secretion inhibited by the embryonic interferon Trophoblastin
4) Target cells
    a. Ovarian luteal cells. PGF2 alpha travels from the uterus to the ovary by traversing a countercurrent diffusion from the uterine vein to the closely apposed ovarian artery and thus into the ovary
    b. Myometrial cells
5) Functions:
    a. Antagonism of luteal cell LH receptors action (cAMP production)
    b. Restriction of CL blood flow
    c. Stimulates oxytocin synthesis and release by the CL
    c. Corpus luteum regression
    d. Increases uterine contractility,

2. Cellular & molecular aspects

A) Hormone secretion occurs principally by exocytosis of secretory granules.

B) Feedback mechanisms; Hormone secretion is regulated by other hormones. Feedback mechanisms include:
1) Inhibition of the production of hormone,
2) Stimulation of the production of hormone,
3) Inhibition of the release of hormone or
4) Stimulation of the release of the hormone.

C) Hormone activity is initiated upon binding of the hormone to a receptor molecule associated with the target cell.

1) Peptide and protein hormones bind to cell surface receptors. Cyclic-AMP is the intracellular messenger for these receptors. Adenylate cyclase; is activated by hormone-receptor complexes or by an intermediary molecule that is activated by the hormone-receptor complex. A typical intermediary is the alpha G protein. When activated the G protein catalyzes the conversion of an attached GDP to GTP. The G Protein-GTP complex then activates adenylate cyclase and converts ATP to cyclic AMP the intracellular mediator of hormone activity. Cyclic AMP stimulates cellular protein production via activation of enzymes or perhaps initiation of transcription. The resulting protein then mediates the activity of the hormone by altering the target cells metabolism and secretory products. In the case of cellular response to a releasing factor or tropic hormone, the resulting secretory product is another hormone.

2) Steroidal hormones, unlike other hormones, have receptors at their target cells that are intracellular. The hormone-receptor complex binds to specific DNA sequences and initiates or modulates DNA transcription and mRNA production. The resulting mRNA is then translated into a protein product.

B. Puberty

1. Age at puberty: The age at puberty varies from species to species. In most species puberty correlates with a rather consistent and species-specific percent of adult body weight. In Man puberty is achieved at about 90% of adult body weight (an unusually high percent). In most species puberty is achieved at 25% to 70% of adult body weight. Age and weight at puberty is considered a valuable economic trait in most livestock species, with early puberty being advantageous. In cattle puberty is achieved at about 65% of adult weight. In horses puberty is achieved at about 75% of adult B.W., sheep at about 50%, most swine breeds at 35%, Chinese pig at 25%, laboratory rats 35%.

2. Stages of puberty; the stages of puberty and physiological mechanisms of puberty are poorly understood. Immature animals characteristically have no pulsatile release of GnRH or pituitary gonadotropins. Even prepubertal aged castrated males have very low FSH and LH levels. Injection of GnRH in a prepubertal animal will stimulate the release of FSH and LH from the pituitary gland and concomitant release of steroidal hormones from the prepubertal gonads. Neither the pituitary nor the gonads seem to be responsible for maintaining the young animal in a prepubertal state. There appears to be a direct CNS suppression of hypothalamic GnRH secretion responsible for maintaining the prepubertal state. Attainment of a critical body size and/or metabolic state (i.e. decrease in rate of growth for instance), or other physical signal probably causes a release of the hypothalamus from the central inhibition.

A) Adrenarche; Secretion of androstenedione from the adrenal glands, is perhaps involved in stimulating the prepubertal growth spurt that most species experience. It may also be involved in some degree of “priming” of the hypothalamus. This occurs several months (most domestic species) to several years (humans) prior to sexual puberty (gonadarche). Adrenal puberty occurs at the same age in males and females.

B) Increase in GnRH pulsatility results in increased circulating levels of FSH and LH. This is responsible for resulting increase in circulating steroidal hormones and in the male a dramatic increase in testicular size. In the female begin to develop beyond the early antral stage and start to produce estrogen and other steroid hormones. Secondary sex characteristics begin to develop at this time. This stage of puberty occurs earlier in females of many domestic species and in man. In males, semen production commences but due to incomplete spermatogenesis fertility remains low.

C) Gonadarche (menarche in women) is heralded by the development and release of viable gametes, the establishment of a functional hypothalamic pituitary gonadal axis, normal estrus cycles in females and ejaculation in males. Gonadarche is more obvious in the female while production of <u>fertile</u> semen should be the main criteria in males.

D) Puberty is a gradual process and development of secondary sex characteristics is the primary noticeable sign of its occurrence.

C. Follicular cycles and estrus cycles

1. Initiation follicular cycles and of estrus cycles at puberty and post-partum. Although different in some respects the basic mechanism of initiating estrus and estrus cycles is similar in most species.

   A) Increase in pulsatile GnRH release stimulates pulsatile gonadotropin release from the anterior pituitary

   B) Increased FSH and LH pulses, low estrogen and progesterone and lack of inhibin allow FSH secretory levels to rise

   C) Follicular cycles are initiated when circulating FSH levels exceeds a threshold of about 100 ng/ml and follicular recruitment or a follicular wave is initiated. (Primates and rodents do not have follicular waves, follicle development is continuous). Initial waves of follicles may not result in a dominant follicle capable of maturation and ovulation if FSH levels are too low (during puberty) or prolactin levels are too high (during lactation). High prolactin causes inhibition of LH release (and perhaps FSH release).

   D) As a competent, dominant follicle or a cohort of graafian follicles in litter bearing animals grow they secretes increasing amounts of estrogen and inhibin, this causes a lowering of FSH secretion

      1) Moderate levels of estrogen may exert a negative feedback on the pituitary and depresses FSH and LH secretion directly but more import estrogen has an inhibitory effect on hypothalamic secretion of GnRH,

      2) Inhibin depresses pituitary FSH production.

      3) Decreased FSH support for non-dominant follicles causes them to undergo atresia. Dominant follicles with active steroid synthesis no longer require FSH to complete maturation.

      4) Late in the follicular stage, higher levels of estrogen have a positive feedback on the hypothalamus, initiating increased pulse frequency and pulse amplitude of GnRH release. This increase in GnRH causes LH release from the pituitary to increase 10 fold resulting in the “LH surge” which is responsible for ovulation. FSH levels also increase 2 to 3 times in response to GnRH.

2. Ovarian follicular cycles and the estrus cycle,

   A) **Proestrus**; Proestrus is the beginning of the "**Follicular Phase**" of the estrus cycle. Proestrus is heralded in all species by elevation of estrogen levels and by growth of a dominant follicles or cohort of graafian follicles. In polyestrus species (cattle, sheep, horses, etc.) proestrus may also be heralded by regression of the previous cycles corpus luteum and a fall in progesterone levels.

   B) **Estrus**; Estrus is heralded by receptivity of the female to mating advances by the male. Events that occur during estrus include:

      1) A peak in estrogen levels at 18 to 36 hours prior to ovulation,

      2) A dramatic elevation in luteinizing hormone levels known as the LH surge occurs. The LH surge occurs at or near the beginning of the true estrus in most species. In cats the LH surge is stimulated by coitus and as a result ovulation will not occur without coitus or some other stimulation.

3) In many species (horses, swine, small ruminants and cats) ovulation of the mature follicle(s) occur at or near the end of estrus. In cattle ovulation occurs 8 to 12 hours after the end of estrus while in the canine ovulation occurs 3 or 4 days prior to the end of estrus.

C) **Metestrus**; is the stage of estrus cycle characterized by decreasing estrogen and increasing progesterone. The female is no longer receptive to male advances. In ruminants, ovulation and fertilization occur at the start of metestrus. The uterus is not yet suitable for the entry of a fertilized embryo and the embryo remains in the oviduct. The length is similar in most species, about 2 to 5 days or until the embryo(s) enters the uterus. True or distinct metestrus does not occur in the canine and metestrus events overlap the last half of estrus.

D) **Diestrus**; is the phase of the estrus cycle where progesterone is elevated and, in a fertile mating, coincides with pregnancy. Progesterone levels are elevated to over 1 ng/ml and the uterus is finally ready to receive the embryo.

E) **Anestrus**; is a period that occurs after a completed pregnancy, during the non-breeding season in seasonally polyestrous species or after a completed estrus cycle in monestrous species. Very low tonic levels of GnRH release from the hypothalamus and low gonadotropin levels characterize anestrus. The entire reproductive system becomes quiescent.