The temporal relationship of the ovary and ovarian morphology for the onset and duratin of behavioral estrus in prostaglandin F☐2a-treated ewes
by Lynn Patricia Courtney

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University
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Abstract:
The objective of this study was to determine the temporal relationship of ovarian follicles and corpora lutea (CL) on the time to onset (TO) and duration (D) of behavioral estrus in PGF2α- treated ewes. Forty normally cycling Western White-Faced ewes were randomly assigned to one of eight treatments to be either sham-operated (SO) or ovariectomized (OVX) at 36, 44, 52 or 60 h after a PGF2α injection given on day 12 of the estrous cycle. At thetime of injection (Oh) and every 4 h afterwards for 96 h , each ewe was observed for estrus using a teaser ram.

At surgery, ovaries of each ewe were macroscopically examined for number and size (mm) of antral follicles and CL. Ovaries of OVX ewes were prepared for histological evaluation. Actual size and number of follicles and CL were determined by taking photographic slides of stained tissue sections and projecting them onto a paper screen. Follicles were classified as atretic (A) or non atretic (NA), and CL as normal or regressing.

Proportion of ewes in estrus was lower (P<.05) for OVX ewes (11 of 20; 55%) than for SO (20 of 20; 100%) and there was a time by treatment interaction (P<.05). Mean TO of estrus was 48.2±3.6 h for OVX ewes which showed estrus and 52.2±2.4 h for SO ewes (P>.05); and the overall mean was 50.7±1.9 h. Duration of estrus was 25.6±.8 h for OVX ewes which showed estrus and 32.6±3.2 h for SO ewes (P>.05); and the overall mean was 29.8±2.5 h. There were no treatment effects for either TO or D of estrus (P>.05). At 44, 52 and 60 h fhe number of actual large NA (LNA) follicles was negatively associated with TO of estrus (r= -.97, -.73, -.87, respectively) and positively associated with D of estrus (r-.76, .70, .65). Actual CL volume was positively associated with D of estrus (r=.84, .58, .68) and negatively associated with TO of estrus (r= -.93, -.36, -.39).

Total number of actual antral follicles did not change over time (P>.05), nor did number of follicles in the small (1-3.9mm), medium (4.0-5.9mm) and large (>6.0mm) size groups (P>.05). There was a shift in class of follicles such that number of medium NA follicles decreased and number of LNA follicles increased from 36 to 52 h (PC.05). CL size was smaller at 60 h (P<.05), while CL volume remained constant (P>.05).

These results indicated that there is a temporal relationship of the ovary for the TO and D of estrus in that the ovary is required for at least 44 h, probably longer, for estrus to occur. However, once estrus has been initiated the presence of the ovary is no longer required for the normal expression of estrus. Individual variation in the TO and D of estrus may be related to differences in the number of LNA follicles and (or) CL volume.

THE TEMPORAL RELATIONSHIP OF THE OVARY AND OVARIAN MORPHOLOGY

FOR THE ONSET AND DURATION OF BEHAVIORAL ESTRUS

IN PROSTAGLANDIN $F_2\alpha$-TREATED EWES

by

LYNN PATRICIA COURTNEY

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This thesis has been read by each member of the thesis committee
and has been found to be satisfactory regarding content, English
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ABSTRACT

The objective of this study was to determine the temporal relationship of ovarian follicles and corpora lutea (CL) on the time to onset (TO) and duration (D) of behavioral estrus in $PGF_2\alpha$- treated ewes. Forty normally cycling Western White-Faced ewes were randomly assigned to one of eight treatments to be either sham-operated (SO) or ovariectomized (OVX) at 36, 44, 52 or 60 h after a $PGF_2\alpha$ injection given on day 12 of the estrous cycle. At the time of injection (Oh) and every 4 h afterwards for 96 h , each ewe was observed for estrus using a teaser ram.

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LITERATURE REVIEW

Introduction

The estrous cycle of the ewe has been studied extensively during the past 20 years. Often the results of these studies have served as a basic model for other domestic species in an effort to understand the mechanisms involved in the reproductive cycle. Although much is known regarding the dynamics of the estrous cycle, there are still many unanswered questions concerning its regulation. A more complete understanding of the interrelationships of factors which govern the estrous cycle of the ewe would result in a more effective control and interpretation of the estrous cycle of domestic ruminants.

The female reproductive process consists of a complex series of hormonal and morphological events involving the ovary, anterior pituitary gland, uterus, hypothalamus and higher brain centers. Physiological regulation of these components involves an interplay of intricate feedback mechanisms of which the timing and responses must be appropriate to ensure a normal estrous cycle. This review summarizes some of the known information concerning the aforementioned relationships and deals specifically with the events occurring during the preovulatory period which pertain directly to this study.

General Characteristics of the Estrous Cycle of the Ewe

Domestic ruminants exhibit a variety of contrasts when comparing events of the estrous cycle. Differences are found in length of the

breeding season, length of the estrous cycle, and time sequence of preovulatory events. One common aspect of the estrous cycle in domestic ruminants is a period of overt sexual behavior (estrus) displayed by the female and expressed as a willingness to be mated by the male.

Breeding Patterns

Ewes are classified as seasonal polyestrus breeders because they exhibit a distinct breeding season comprised of several estrous cycles and a non-breeding (anestrus) season (Wiggins et al., 1970). In contrast, the cow and sow are considered polyestrus and do not exhibit pronounced periods of anestrus (Cupps et al., 1969). The anestrus period of the ewe occurs during days of increasing daylight, which in the northern latitudes usually extends from March through August (Lees, 1965; Shackell et al., 1977). Anestrus is characterized by the absence of behavioral estrus and suppression of endogenous reproductive activity (Brinkley, 1981; Yuthasastrakosol et al., 1975).

Estrous Cycle Length and Duration of Estrus

In the ewe a great deal of variation has been reported in length and duration of the estrous cycle within breeds (Wiggins et al., 1970), among breeds (Hanrahan and Quirke, 1974) and between sheep in different geographical locations (Hafez, 1952). On the average, for ewes in the northern hemisphere, the breeding season, or period of maximum sexual activity, occurs during November through January (Hafez, 1952). An average period of 15 to 17 days elapses from the onset of one behavioral estrus to the next and duration of estrus generally ranges from 36 to 48 h (Cupps et al., 1969).

Phases of the Estrous Cycle

The estrous cycle can be divided into two distinct phases; luteal and follicular phases. In the ewe, the luteal phase is approximately 12 to 13 days in duration and is dominated by the presence of the corpus luteum (CL) in the ovary and its hormonal secretory product, progesterone. The follicular phase spans 3 to 4 days and is characterized by a decline in progesterone and a rise in estrogen and gonadotropin secretion, rapid follicular growth, a period of behavioral estrus, a preovulatory gonadotropin surge and culminates with ovulation (Legan and Karsch , 1979).

Regulation of Cyclical Changes in Ovarian Morphology and Cytology of the Ewe

During each estrous cycle the ovaries undergo a series of structural and functional changes involving the growth and disappearance of CL and follicles. Structurally, the ovary consists of an inner medullary region containing blood vessels, lymphatics, nervous tissue, and dense connective tissue which enter the ovary at the hilus, or point of attachment to the suspensory ligament. Connective tissue surrounds the surface of the ovary forming a capsule or tunica albuginea. Under the capsule lies the cortex in which are located the CL and follicles (Johnson, 1982; Deane, 1952).

Follicular Growth and Development

Follicles exist in either a non-growing or growing phase. Those follicles that are in the growth phase can be seen in various stages of development and in all size classes throughout the cycle. In fact,

the number of follicles in each size class tend to remain constant at all times creating a balance between the number of follicles entering the growth phase, growth into the various size classes, and those lost to atresia (follicular death; Hay and Moor, 1975; Brand and Jong, 1973).

There are some subtle changes in the follicular population during the preovulatory period. In the ewe, the total number of follicles remains the same but tends to increase in size (Kammlade et al., 1952), while in the cow it seems that the number of small follicles decrease while the population of large follicles turn-over more rapidly (Matton et al., 1981).

Kinetic studies of follicular development revealed three waves of follicular growth and atresia per estrous cycle in the ewe (Smeaton and Robertson, 1971) as evidenced by fluctuating levels of estrogen as follicles grow and degenerate (Baird and McNeilly, 1981). Waves of follicular growth occur during the early luteal phase (days 6 to 9) and late luteal phase (days 13 to 15) with all of these follicles becoming atretic, and finally during the preovulatory period in which a follicle or follicles eventually ovulate (Brand and Jong, 1973; Hauger et al., 1977).

Stages of Follicular Development. Primordial follicles constitute the smallest size class and are considered as the pool of non-growing follicles from which growing follicles are selected. Primordial follicles consist of an oocyte surrounded by a single layer of flattened epithelial cells, or granulosa cells (Rajakoski, 1960). Stroma cells immediately surrounding the follicle will later become

theca cells as the follicle develops (Deane, 1952; Johnson, 1982).
When primordial follicles enter the growth phase, granulosa cells
become activated and these cells multiply forming a multilayered
membrana granulosa. This layer in cooperation with the oocyte form
the zona pelucida, a thin membrane which separates the two structures.
The developing theca cells begin to differentiate and take on cellular
characteristics indicative of steroid secreting cells. During this
process the oocyte enlarges and the follicle begins to migrate towards
the medulla (Johnson, 1982).

As the follicle continues to enlarge, small cavities begin to
appear within the membrana granulosa. These cavities coalese to form
an antrum which expands and becomes filled with follicular fluid
produced by granulosa cells (Johnson, 1982). Follicles of this type
range in size from 1 to 6mm in diameter in the ewe (Armstrong et al.,
1981).

The largest size class consists of the Graafian or preovulatory
follicle which contains a large single chambered antrum, a thick
granulosa layer, and a fully differentiated theca interna and externa
(Rajakoski, 1960). The oocyte is pushed to one side of the antral
chamber by follicular fluid and continues to be surrounded by several
layers of granulosa cells identified as the cumulus oophorus. Such
follicles appear as fluid filled blisters on the surface of the ovary
(Johnson, 1982). In the mature ewe, 12,000 to 86,000 small follicles
(<2 layers of granulosa cells) and 100 to 400 larger follicles are
present throughout the estrous cycle (Cahill et al., 1979) and these
numbers diminish with age (see review by Cahill, 1981).

Microscopic Classification of Follicles. Once follicular growth
has begun, follicles are committed to either mature to ovulation or
become atretic (Peters et al., 1975; Richards and Midgley, 1976). At
any one time during the estrous cycle approximately 32% of the growing
follicular population is found to be in a normal developing state; the
remaining 68% are found in some stage of atresia (Brand and Jong,
1973). Ultimately, 99% of all follicles that enter the growth phase
become atretic (Richards, 1980).

Growing antral follicles show a theca interna layer composed of
fibroblast-like cells with collagen fibers arranged between cells. As
estrus approaches, thecal cells increase in number and size and this
layer becomes highly vascularized. Characteristic of steroid
secreting cells, they contain secretory granules, an abundance of
smooth endoplasmic reticulum, well developed golgi bodies, a limited
amount of lipid droplets and steroid-type mitochondria. On the other
hand, granulosa cells show characteristics of protein secreting cells.
They contain rough endoplasmic reticulum, well developed golgi bodies
and many free ribosomes (Hay and Moor, 1975). The cumulus oophorus is
actively mitotic during the growth phase (Hay et al., 1976).

As follicles become atretic they lose their bright, uniform
appearance and exhibit signs of degeneration (Moor et al., 1978). The
stages of atresia have been described in detail by Brand and Jong
(1973), Hay et al. (1976) and Mauleon (1969). Early stages involve
the termination of mitotic activity in theca and granulosa layers, and
pycnotic nuclei are prevalent in granulosa cells surrounding the
antral cavity. The number of pycnotic cells continue to increase and

soon dark staining atretic bodies (aggregates of nuclear material) appear. Some phagocytes are present and destruction of cells around the oocyte begins. In the later stages of atresia the granulosa layers have thinned considerably and the cells begin to disassociate and can be found floating freely in the antral fluid. The follicular wall becomes distended and begins to fold back on itself. In contrast to the extensive damage to the granulosa layer, the theca layer remains virtually unchanged.

Hormonal Regulation of Follicular Development

The factors involved in the initiation of follicular growth have yet to be determined but it does not appear that gonadotropins are essential (Baird and McNeilly, 1981; Cahill, 1981). For both the mouse (Peters, 1979) and rat (Schwartz et al., 1974), exogenous gonadotropins did not increase the number of follicles entering the growth phase and anti-gonadotropins did not decrease the number. It has been suggested that factors produced by surrounding growing follicles or atretic follicles may stimualate primordial follicles to enter the growth phase (Peters et al., 1975). Once activated, any further growth of the follicle requires hormonal stimulation (Dufour et al., 1979).

Preantral Stage. Once primordial follicles enter the growth phase a period of time, up to 6 months, may be required until the preovulatory stage is reached in the ewe (Cahill and Mauleon, 1980), opposed to only 20 to 30 days in the mouse (Peters and Levy, 1966). The earliest stages of follicular growth require stimulation by follicle stimulating hormone (FSH) and estrogen to initiate

proliferation of the granulosa cells and overall growth of the follicle (Harman et al., 1975). The role of luteinizing hormone (LH) in early follicular development is not clearly understood but is believed to be involved in differentiation of theca cells (Gibori and Miller, 1982). During the preantral stage atresia is rare and follicles generally remain dormant up to 4 months (Cahill and Mauleon, 1980).

Antral Stage. Reactivation of dormant preantral follicles is triggered by some unknown mechanism (Smeaton and Robertson, 1971), possibly by a preceding preovulatory gonadotropin surge (Goodman et al., 1981b). Once stimulated, FSH promotes antral formation (Evans et al., 1932), increases the number of follicular LH (Zeleznik et al., 1974) and FSH receptors (Gibori and Miller, 1982) and acts with estrogen to stimulate rapid follicular growth (Richards and Midgley, 1976). Luteinizing hormone enhances the action of FSH in increasing the number of LH receptors in granulosa cells (Gibori and Miller, 1982).

Production of estrogen by antral follicles requires the synergistic action of LH and FSH (Armstrong et al., 1981). Moor (1973) explanted antral follicles >2mm in diameter from ewes at various times during the estrous cycle, maintained them in organ cultures and monitored estradiol output. He found that small and medium follicles (<4.5mm) sercete low amounts of estradiol, with most estradiol production coming from 1 or 2 large follicles; the amount produced was dependent on stage of the estrous cycle. The work of several investigators, as reviewed by Richards and Midgley (1976),

have shown that estrogen increased the sensitivity of granulosa cells to LH and FSH stimulation, thereby increasing its own systhesis. Under LH stimulation theca cells produce androgens which granulosa cells under LH and FSH influence aromatize to estrogen (Armstrong et al., 1981; Richards, 1980). The LH surge blocks further estrogen production by interfering with the aromatase enzyme system that converts androgens to estrogen (Baird et al., 1981). Therefore estrogen production appears to be regulated not only by gonadotropins but intrafollicular estrogen as well.

Preovulatory Stage. Development of follicles from the antral to preovulatory stage requires approximately 34 to 43 days in the ewe (Cahill, 1981). The mechanism for selection of the follicle(s) to ovulate have not been elucidated. Harman et al. (1975) demonstrated in hypophysectomized rats treated with anti-estrogen, that FSH and LH alone increased the percent of atretic follicles while estrogen reduced the number of atretic follicles. It is possible follicles are eliminated from the non-atretic pool via local effects of gonadotropins and estradiol as they work in oppossition to one another in regulating follicular growth and atresia.

In response to the preovulatory gonadotropin surge the selected follicle(s) undergo final maturational changes in preparation for ovulation which include production of proteolytic enzymes to weaken the follicular wall (Thibault et al., 1975) and an accumulation of prostaglandins E and F in antral fluid (Bauminger and Linder, 1975) which are presumably involved in the eventual rupture of the follicle.

Atresia. The mechanism by which follicles become atretic has not been firmly established. There is some indication that gonadotropins may be responsible for atresia in the rat. Harman et al. (1975) treated hypophysectomized rats with estrogen and found the growth rate of follicles to increase with no increase in the atretic rate. When anti-estrogen was used concurrent with exogenous FSH and LH, it was observed that the total number of follicles increased but a greater percentage of them were atretic.

Reviews by Carson et al. (1979) in the ewe and Richards and Midgley (1976) in the mouse, describe the response of follicles to gonadotropins and their possible relationship to atresia. Preantral folicles require LH and FSH to grow but apparently do not respond to gonadotropin surges. Larger antral follicles may respond to a gonadotropin surge positively (ovulation) or negatively (atresia), or they may become atretic prior to the gonadotropin surge. It has been proposed that antral follicles develop a mechanism (ie., increased LH receptors) or attain a sensitivity to the LH surge at some time during their development. If this mechanism is developed too early follicles may become atretic since they were not be exposed to the LH surge. If the surge occurs and the follicles have not attained sensitivity to gonadotropins, this may also lead to atresia. From this hypothesis it can be assumed that if a follicle is to remain in the growing phase it must retain sensitivity to LH and FSH and the growth pattern of the follicle must be properly synchronized with the preovulatory gonadotropin surge in order to ovulate. Atresia may also be caused by changes in permeability of the basement membrane which allows serum

factors to reach the granulosa cells and cause an inflammatory response which destroys these cells (Farookhi, 1981). Louvet et al. (1975) found that androgens increase the number of atretic follicles observed in a population. Therefore, follicles that produce excess androgens or are unable to convert androgens to estrogens may be more likely to become atretic.

Corpus Luteum Development and Function

The CL is subject to stages of growth and degeneration similar to that of ovarian follicles. Following ovulation of the preovulatory follicle there occurs a transformation of the surrounding follicular cells into luteal cells. The process of luteinization is not necessarily preceded by ovulation (MacKenzie and Edey, 1975) but is initiated by the LH surge. A review by Gibori and Miller (1982) describes the mechanism of luteinization in rodents which involves the loss of LH, FSH and estrogen receptors from the theca and granulosa cells of the follicle, differentiation of these cells to luteal cells, and finally the reappearance of the receptors necessary for the production of progesterone. In the ewe the corpus luteum remains functional from days 2 through 14 of the estrous cycle (Hansel et al., 1973).

Microscopic Classification. In the ewe, cow, and sow the CL is formed from the membrana granulosa and theca interna of the ovulated follicle (Hansel et al., 1973). These cells differentiate into granulosa lutein cells forming a thick inner region often folded in appearance, and theca lutein cells which constitute the thinner outer

layer (Cupps et al., 1969) and a central cavity may or may not be present (Deane et al., 1966).

In the functional CL, granulosa lutein cells are easily identified as pale staining, large ployhedral cells with abundant, granular cytoplasm. Numerous mitochondria are present in various shapes and sizes, smooth endoplasmic reticulum is more common than the rough variety (Enders, 1973; Deane et al., 1966) and secretory granules have been observed (Gemmell et al 1976). Theca lutein cells are smaller and the nuclei stain darkly. This area is rich in capillaries which invade the granulosa layer along with connective tissue (Peters and Levy, 1966; Leeson and Leeson, 1979).

During luteal regression, cells of the granulosa layer shrink and lose their shape. Their nuclei stain darkly, vacuoles appear, vascularization diminishes and lysosomes become more fragile. Steroid systhesis fails as indicated by the accumulation of lipid droplets and the decline in secretory granules. The CL migrates away from the periphery of the ovary as it shrinks in size, and connective tissue becomes manifest throughout. Soon only scar tissue remains and the structure is referred to as a corpus albicans (Gemmell et al., 1976; Hansel et al., 1973; Deane et al., 1966; Leeson and Leeson, 1979; Peters and Levy, 1966; Deane, 1952).

Hormonal Regulation of Corpus Luteum Function. The absolute requirements to maintain luteal integrity in the ewe have not been determined. It seems clear that LH at least is required. Kaltenbach et al. (1968) hypophysectomized cyclic ewes and observed a rapid degeneration of the corpus luteum. Crude pituitary extracts

containing FSH, LH, prolactin and other hypophyseal hormones were found to maintain the corpus luteum. Crude LH preparations were partially luteotropic while replacement of other pituitary hormones had no maintenance effects. Prolactin may play a permissive role based on the fact that serum prolactin levels increase in those ewes in which the CL was being maintained with LH infusions in partially hypophysectomized ewes (Schroff et al., 1971).

The functional activity of the CL, or progesterone systhesis, appears to be regulated by gonadotropins in the ewe. Cook et al. (1969) demonstrated that progesterone synthesis by sheep CL *in vitro* was stimulated when exposed to LH. Hixon and Clegg (1969) found that in hypophysectomized ewes, both LH and prolactin had the capacity to stimulate progesterone secretion.

In the ewe, CL function is thought to cease due to the action of some luteolytic factor(s) secreted by the uterus (Goding, 1974). Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) has been implicated as a major luteolytic factor in the ewe (Goding, 1974). Several conditions must exist before luteolysis can occur. First, the uterus must be exposed to elevated levels of progesterone for at least 7 to 10 days before it can systhesize sufficient quantities of $PGF_2\alpha$ (Baird, 1978b). Tonic levels of LH must be present throughout the luteal phase to increase the sensitivity of the CL to $PGF_2\alpha$, and low levels of estradiol are required to stimulate the release of $PGF_2\alpha$ (Baird and McNeilly, 1981; Goding, 1974; Hixon et al., 1975) or its precursors (Gibori and Miller, 1982; Hansel et al., 1973) from the uterus. It has been suggested, in the cow at least, that endometrial secretions contain

precursors which the CL is capable of converting to $PGF_2\alpha$. Developing

follicles may also play a role in the destruction of the CL via direct

competition for available LH, which is required for luteal maintenance

(Karsch et al., 1970).

Several methods have been proposed regarding the question of how

$PGF_2\alpha$ initiates luteal regression. It is possible that $PGF_2\alpha$ exerts

is effects in either one or a combination of the following methods; 1)

decreasing the blood flow to the ovary bearing the CL (Nett et al.,

1976), 2) changing the structure of the phospholipid bilayer of the

luteal cell membrane resulting in the loss of tissue function (Buhr et

al., 1979) and(or) 3) weakening lysosomes of luteal cells allowing the

release of enzymes which ultimately destroy these cells (Stacy et al.,

1976).

Obvious advantages in management can be obtained by using $PGF_2\alpha$

as an estrus synchronizing agent. Through widespread experimentation

for use in cattle and sheep operations, some interesting observations

have been made concerning the varying individual resposes to the

luteolysin. For example, a group of ewes given unequal doses of $PGF_2\alpha$

displayed varying degrees of luteal regression and decreases in

circulating levels of progesterone (Stacy, et.al., 1976) suggesting a

minimum dose requirement. Also of interest is the time it takes for

an individual to respond to $PGF_2\alpha$ and come into estrus. Chamely et

al. (1972) administered equal doses of $PGF_2\alpha$ to ewes with ovarian

transplants and observed a span of 42 to 66 h afterwards in which the

ewes came into estrus. The reason for this variation has not been

explained and it is not clear whether it is due to differences in the

rate of decline of progesterone after the $PGF_2\alpha$ treatment or due to other extraovarian factors.

Hormonal Regulation of the Estrous Cycle of the Ewe

The signals and responses which dictate the temporal pattern of events which comprise the estrous cycle are mediated via hormones. With the aid of highly sensitive radioimmunoassay techniques and surgical ablation of tissues or organs with selective replacement of their secretions, the complex patterns of regulation were gradually revealed. From this information, working models have been constructed, although to date there are still many gaps in our understanding.

Hormonal Patterns of the Luteal Phase

During the luteal phase the major ovarian steroid found in high concentrations in the blood is progesterone, known to reflect the functional activity of the CL in the ewe (Stormshak et al., 1963). Progesterone secretion from the newly formed CL steadily rises from day 2 (day 0=estrus) to approximately day 9 when peak concentrations are reached. Serum levels remain elevated until day 16 when they begin to decline due to luteal regression. By day 17 progesterone has fallen to basal levels (Cunningham et al., 1975; Stabenfeldt et al., 1969)

Estrogen is also produced during the luteal phase, although in limited quantities. The source of estrogen is exclusively from large antral follicles of about 5mm in diameter in both the ewe and cow (Baird et al., 1981; Baird et al., 1975; Karsch et al., 1970), whereas

in primates, the CL is also capable of estrogen production (Baird et al., 1975). In the ewe, 3 peaks of estrogen secretion are observed. Aside from the preovulatory phase increase, two peaks occur during the luteal phase; between 4 to 6 days and 9 to 11 days after the LH surge. For the remainder of the luteal phase estrogen levels decline (Hauger et al., 1977).

The pattern of LH secretion fluctuates during the luteal phase. Concentrations of LH are still high during the early luteal phase from the preovulatory surge and gradually decline so that by day 6 to 8 following the LH surge basal levels are reached. A gradual increase in LH secretion is again observed during the late luteal phase, 2 to 3 days prior to the LH surge (Hauger et al., 1977). Initially FSH follows the pattern of LH secretion by gradually declining from preovulatory surge concentrations to basal levels. Before presurge baseline values are reached FSH secretion rises slightly and then either declines steadily or in a series of small peaks (Goodman et al., 1981a).

Progesterone and Estrogen: Negative Feedback on LH and FSH. Experimental data has accumulated that indicate that levels of LH during the luteal phase, described as tonic secretion, are under some type of inhibitory control. Ovariectomy results in an increase in LH and FSH levels in the blood, indicating the ovary has an inhibitory action on gonadotropin release, as shown in the ewe (Howland and Stormshak, 1969) and rat (Grady and Schwartz, 1981). Karsch and Legan (1978) ovariectomized ewes during the mid luteal phase, observed the rise in circulating gonadotropins, and selelectively replaced estrogen

and progesterone at luteal levels either alone or in combination to study their effects on LH secretion. They found that either steroid alone only partially restored LH levels, the full inhibitory effect was achieved when both steroids were replaced. Goodman et al. (1980) performed a similar experiment and found gradual replacement of progesterone simulating luteal phase concentrations was the dominant force in LH inhibition. Simultaneous administration of estradiol augmented the inhibitory effect of progesterone.

Also of interest is work done by Roche et al. (1974). Serum LH was monitored in ewes following the sequential removal of antral follicles (source of estradiol), CL (source of progesterone) and stroma during the luteal phase. In the absence of follicles LH levels remained low. When only the CL was destroyed, LH levels exhibited a partial increase. When both follicles and CL were removed LH levels escalated similar to that seen after a bilateral ovariectomy. In summary, it seems progesterone is the primary control during the luteal phase in maintaining tonic secretion of LH. Estradiol from antral follicles serves to enhance this effect.

Regulation of Estrogen Secretion. Excluding the two rises in estrogen observed during the luteal phase, for the most part concentrations remain at basal values. It has been established that follicular development and estradiol secretion are highly dependent on LH stimulation (Armstrong et al., 1981). When progesterone levels are elevated, LH secretion is inhibited and the rate of estradiol secretion was reduced (Hauger et al., 1977). In essence, progesterone indirectly inhibits estrogen secretion by maintaining basal levels of