



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

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
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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 α 2-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 α 2 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.

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 $25.6 \pm .8$ 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 the 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 (> 6.0 mm) 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 ($P < .05$). 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.

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FOR THE ONSET AND DURATION OF BEHAVIORAL ESTRUS
IN PROSTAGLANDIN F₂^α-TREATED EWES

by

LYNN PATRICIA COURTNEY

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of

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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 $\text{PGF}_2\alpha$ -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 $\text{PGF}_2\alpha$ injection given on day 12 of the estrous cycle. At the time of injection (0h) 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 coalesce 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 Mauleón (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 stimulate 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 (Zelevnik 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) secrete 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 synthesis. 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 opposition 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 follicles 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 (i.e., 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 polyhedral 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 synthesis 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 synthesis, 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 synthesize 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 $\text{PGF}_2\alpha$. 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 $\text{PGF}_2\alpha$ initiates luteal regression. It is possible that $\text{PGF}_2\alpha$ exerts its 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 $\text{PGF}_2\alpha$ 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 responses to the luteolysin. For example, a group of ewes given unequal doses of $\text{PGF}_2\alpha$ 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 $\text{PGF}_2\alpha$ and come into estrus. Chamely et al. (1972) administered equal doses of $\text{PGF}_2\alpha$ 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 $\text{PGF}_2\alpha$ 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 selectively 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

LH and decreasing the amount of LH available to stimulate ovarian follicles.

Hormonal Patterns of the Follicular Phase

Initiation of luteolysis and the resulting decline in progesterone secretion mark the beginning of the follicular phase. Concomitant with this event is an increase in the pulsatile release of LH thought to be the cause of increased estrogen synthesis and secretion (Baird and McNeilly, 1981). However, recent evidence indicated that the initial increase in estrogen after the fall in progesterone is not influenced by changes in LH concentrations (Gust et al., 1984). The increase in estrogen secretion appears to begin on day 15 and is thought to be a function of maturing preovulatory follicles (Bjersing et al., 1972). Estrogen and LH levels continue to rise in a parallel fashion until LH reaches some "critical" concentration, then estrogen levels begin to decline (Baird, 1978a). Follicular phase levels of estrogen serve both neuroendocrine and neural functions; 1) to induce a period of behavioral estrus (Karsch et al., 1980; Robertson, 1969) observed approximately 24 h after the completion of luteal regression (Stabenfeldt et al., 1969), and 2) to stimulate the preovulatory LH surge (Legan and Karsch, 1979; Fairclough et al., 1976). At estrus, LH reached values of 40 ng/ml with maximum preovulatory surge concentrations up to 250 ng/ml occurring 1 to 24 h after the onset of behavioral estrus (Hauger et al., 1977). Ovulation takes place 36 to 40 h after the onset of estrus (Cupps et al., 1969) or approximately 21 to 26 h after the LH peak (Cumming et al., 1973).

Estrogen: Positive Feedback on LH. Luteinizing hormone is known to be released in a pulsatile fashion from the anterior pituitary gland and Baird (1978a) reported the frequency of LH pulses increased from 1 pulse per 3 h and 20 min during the luteal phase to 1 pulse per h in the follicular phase. At estrus a rate of 1 pulse per 45 min or less was observed. While the pulse frequency of LH increased, the amplitude decreased, and each pulse was followed by a greater increase in estradiol secretion as the levels of both hormones continued to rise in parallel.

The rapid decline in estradiol secretion around the time of the LH surge in the ewe probably can not be attributed to ovulation since ovulation usually follows 24 h after the LH peak (Cumming et al., 1973). Moor (1974) proposed that the preovulatory LH surge terminated estrogen secretion by acting directly on the follicle, altering its function from estradiol to progesterone production. He demonstrated this by exposing follicles maintained for 7 days in a culture medium with preovulatory-like concentrations of LH. Prior to LH treatment the follicles produced high levels of estradiol and little progesterone. Addition of LH resulted in a rapid decline in estradiol and a significant increase in progesterone secretion. He obtained similar results in vivo with ewes that had had the CL and ovary without the largest follicle removed. Infusions of LH 18 h after surgery prevented the rise in estrogen production that would have been expected and progesterone secretion was elevated.

The preovulatory surge pattern of estradiol is important in regulation of LH secretion. Fairclough et al. (1976) and Ferin et al.

(1969) have shown in the ewe and rat, respectively, that by blocking the estrogen surge with antibodies to estradiol-17 β , it resulted in blocking the LH surge and ovulation. Maintaining estrogen levels exogenously after levels would normally decline diminished the amplitude of the LH peak (Turgeon, 1979). These results demonstrate a time course relationship of estrogen secretion and its importance in affecting a proper LH surge pattern.

Although estrus (Scaramuzzi et al., 1971) and ovulation (Wheeler et al., 1977; Goding et al., 1970) have been induced in the ewe with exogenous administration of estradiol, the exact concentration required, if any, and the variation of this level in vivo has yet to be determined. The time course requirement for the presence of the ovary in the initiation of preovulatory events should be addressed. It has been shown that once the LH surge has begun, the presence of the ovary is no longer required for a normal LH surge in the ewe (Webb et al., 1981), yet similar experiments for the onset and duration of estrus have not been reported. In order to elucidate whether a threshold value of estrogen in vivo is necessary and to determine the role of the ovary, ovariectomy performed at predetermined intervals prior to estrus, evaluation of serum patterns of LH, and observation for signs of behavioral estrus should be performed. Another method might be to selectively replace varying levels of estradiol in progesterone-treated, ovariectomized ewes to determine the in vivo threshold value.

Regulation of FSH. Control of FSH secretion is less clear due to the lack of adequate assay techniques making it difficult to monitor

FSH release patterns. However, it is apparent that replacement of estrogen or progesterone in the ovariectomized ewes does not play a crucial role. It has been proposed that another ovarian factor may be responsible for regulating FSH patterns, since replacement of estrogen and progesterone did not restore normal FSH patterns (Goodman et al., 1981a). A protein identified as inhibin has been isolated from granulosa cells and follicular fluid and has been shown to directly inhibit FSH synthesis and secretion (Franchimont et al., 1981) and may be the suspected ovarian factor.

Summary: Role of Progesterone and Estrogen. It is important to realize that progesterone and estrogen act in concert throughout the estrous cycle. While estrogen is responsible for the quantity of LH released, it is progesterone that ensures its release at the proper time and for the proper duration, as shown in the ewe (Karsch et al., 1980) and hamster (Norman, 1975). In the ewe it has been demonstrated that when preovulatory surge levels of estradiol were insufficient, estrus and ovulation became asynchronous. Proper amplitude of estrogen coupled with prior exposure to progesterone and its withdrawal, assures the normal synchronization of estrus and ovulation (Goodman, 1978). From these studies it is evident that concerted actions of estrogen and progesterone are essential for the normal occurrence of preovulatory events.

Role of the Central Nervous System (CNS)

There is evidence to support the idea that in the ewe, progesterone and estrogen act at the level of the hypothalamus (CNS) and anterior pituitary gland in feedback control of LH and FSH

release. Goding et al. (1970) demonstrated in ovariectomized ewes that estradiol controls tonic LH secretion and is required to induce the LH surge. This relationship seems to indicate tonic LH secretion and preovulatory surge release are controlled by two separate centers. The hypothalamus certainly plays a role in the preovulatory surge since immunization of ewes to gonadotropin releasing hormone (GnRH), produced by the hypothalamus, blocks the LH surge (Fraser and McNeilly, 1982). The CNS is further implicated, in that induced LH surges in ovariectomized ewes are blocked with barbituates and can be restored by injecting GnRH (Radford and Wallace, 1974).

To determine the mechanism of estradiol in LH release, Coppins and Malven (1976) injected preovulatory levels of estradiol into ovariectomized ewes. An 8 h period of LH inhibition was followed by an LH surge 12 to 20 h after treatment. The experiment was repeated with the addition of GnRH given in a periodic or pulsatile pattern. An initial 4 h period of pituitary insensitivity to GnRH was observed, with sensitivity reestablished by 6 h, and a hypersensitive condition existing by the onset of the LH surge. Similar results have been observed in cattle (Kesner et al., 1981). These results suggest estradiol-induced LH depression is due in part to pituitary insensitivity to GnRH which is restored approximately 6 h after estradiol treatment, and inhibition of GnRH release which is not reversed until approximately 8 h after estradiol treatment.

Brain lesion techniques have been employed to identify the specific areas in the CNS involved in regulating LH secretion. Jackson et al. (1978) severed neural afferent connections through the

suprachiasmatic nuclei (SCN) and preoptic area (POA) in the ewe and found LH surges were eliminated while basal LH release patterns were unaltered. In the rat, the LH surge is prevented by severing the SCN (Hayashi and Mizukami, 1979; Rush et al., 1980) and initiated when the POA is electrochemically stimulated (Terasawa and Sawyer, 1969). Regulation of tonic LH secretion appears to be located in the medial basal hypothalamus (MBH) in the ewe (Prezko and Domanski, 1980) and rat. Blake (1977) severed all neural input to the MBH in ovariectomized rats, replaced estrogen and progesterone and found basal LH levels were maintained. Doses of GnRH resulted in increased LH release indicating inhibition at the MBH level is by preventing GnRH release.

In summary, it appears estradiol initially decreases pituitary sensitivity to GnRH and inhibits GnRH release from the hypothalamus, followed by a reversal of these effects at the time of the LH surge. The LH surge is triggered by the action of estradiol on the POA and SCN centers of the brain. Tonic LH secretion is regulated by progesterone and estradiol inhibition of GnRH release from the medial basal hypothalamus.

Regulation of Sexual Behavior in the Ewe

Sexual behavior is an important part of reproduction for it is the signal that brings the male and female together to mate. In the ewe, sexual behavior (estrus) is expressed in various ways. Ewes tend to follow the male, actively compete with other estrous ewes for the

ram's attention, and are the aggressor in seeking out the male to stand and be mated (Hulet et al., 1962; Anderson, 1969).

Hormonal Control

Essential to estrous behavior is a decline in luteal levels of progesterone followed by increased concentrations of estradiol (Short, 1972). Behavioral estrus can be induced in ovariectomized ewes with estradiol alone, but the behavior pattern is abnormal. Prior conditioning with progesterone restored normal estrous behavior (Robinson, 1954). It has been postulated for the ewe (Scaramuzzi et al., 1972) that progesterone heightens the sensitivity of the hypothalamus, thus lowering the threshold requirement of estradiol needed to induce estrus. If progesterone conditioning is absent, higher levels of estradiol are required to trigger behavioral estrus. The opposite condition exists in the rat. For lordosis to occur, the rise in estradiol must precede the increase in progesterone (Barfield and Lisk, 1970). The apparent role of estradiol is to prime the hypothalamus (increase the number of progesterone receptors) to respond to rising levels of progesterone, which induce lordosis (Parsons et al., 1980, Roy et al., 1979). In the ewe, the role of ovarian steroids may be the reverse, in that progesterone increases the number of hypothalamic estradiol receptors that respond to rising estradiol levels to trigger estrus.

Brom and Schwartz (1968) demonstrated a time requirement of the ovary for lordosis in rats. If ovariectomy was performed on the morning of expected lordosis the behavioral period was early and shortened, indicating threshold levels of progesterone and estradiol

were not yet reached. Also, there appears to be a critical duration of estradiol exposure in the rat, where the hypothalamus requires a minimum of 30 minutes of elevated estradiol stimulation to induce normal lordosis (Johnston and Davidson, 1979). It is known that a critical level of estradiol is required in the ewe (Goodman, 1978), but a minimum duration of estradiol exposure has not been reported.

Hanrahan and Quirke (1974) observed several breeds of sheep and found that duration of estrus was greater in breeds with higher ovulation rates. A possible explanation for this difference is that ewes with higher ovulation rates may be producing greater quantities of estradiol, which has been shown to increase duration of estrus in ovariectomized ewes (Land et al., 1972).

Role of the Central Nervous System

Because estrus is a behavioral phenomenon it is logical to assume the CNS is involved. The most convincing evidence for this is supported by brain lesion experiments with rodents. Brookhart et al. (1941) provided some early insight by performing hypothalamic lesions on guinea pigs. All animals were unable to display estrus while most continued to have normal ovarian cycles. This provided evidence that the CNS was involved but the precise site of action was unknown.

Since then, more refined techniques have been developed and the role of the CNS has become more clearly defined. The ventro-medial hypothalamus (VMH) has been shown to have an unusually high affinity for estradiol (Dohanich and Clemens, 1981) and estradiol implants to this area have the greatest effect on inducing lordosis in rats (Barfield and Chen, 1977). The VMH is also believed to be the center

of estrus behavior in the ewe, since lesions in this area abolish estrus while other facets of the estrous cycle remain intact (Clegg et al., 1958).

In conclusion, for a normal display of behavioral estrus in the ewe, a prolonged period of luteal levels of progesterone is required to increase the sensitivity (i.e. possibly to increase estradiol receptor numbers) of the VMH to elevated levels of estradiol present after luteal regression. The VMH responds to some "threshold" level of estradiol to trigger a period of overt sexual behavior. Individual variation in the estrous response could be due to differences in the number estradiol receptors in the hypothalamus, the threshold level of estradiol needed to induce behavioral estrus, the levels of estradiol the hypothalamus is exposed to and (or) the duration of exposure to elevated estradiol.

STATEMENT OF THE PROBLEM

In species exhibiting estrous cycles, such as the ewe, behavioral estrus and ovulation are closely related events regulated by neural and hormonal mechanisms. The feedback effects of estradiol and progesterone acting at the level of the hypothalamus and anterior pituitary gland regulate gonadotropin secretion and sexual behavior throughout the cycle.

Various observations and experimental results have led to the concept that behavioral estrus and preovulatory LH secretion are controlled by two separate mechanisms. The occurrence of silent heat observed in the ewe, cow, and sow provides supporting evidence. Although silent estrus is not seen in the rat, estrus and ovulation can be induced separately with varying doses of estrogen. However, the most convincing evidence, has been provided through utilization of brain lesion techniques which implicate the SCN and POA as the gonadotropin surge centers and the VMH as the center for estrous behavior in both the rat and ewe.

Estrus and the preovulatory LH surge have been induced in the ewe with exogenous administration of estradiol, yet the in vivo threshold requirement is unknown. It is known that the decline in progesterone and follicular development at the end of the estrous cycle with respect to estradiol production, is highly correlated with estrus behavior. The level of estradiol production is important since greater amounts of estradiol have been shown to increase the duration of estrus in ovariectomized ewes. It has been observed that estradiol

levels begin to decline by the time of estrus onset suggesting continuous estradiol production is not required for the normal expression of estrus, therefore a temporal relationship may exist between the ovary and estrous behavior.

Studies related to dynamics of follicular growth in the ewe revealed that although the total number of follicles do not change with stage of the estrous cycle, size of follicles increased towards the end of the estrous cycle as estrus approaches. It has also been observed that ewes with higher ovulation rates, and therefore a greater number of large non-atretic follicles during the preovulatory period, have a longer period of behavioral estrus. A delay of 42 to 66 h is often observed in the ewe in relation to the time to onset of estrus following a $\text{PGF}_2\alpha$ injection. It is possible that this delay, as well as differences in the duration of estrus may be attributed to a particular follicular population at the time of luteal regression. There may be individual differences among ewes for the number of follicles responding to rising levels of LH by increasing in size and estradiol production. Ewes that develop a greater number of large follicles quickly, following either natural or induced luteal regression, may come into estrus earlier and for a greater duration than ewes with fewer large follicles that develop more slowly and produce less total estradiol.

The intent of this study was to focus on regulation of behavioral estrus, specifically to determine the temporal requirement of the ovary and to evaluate the role of the CL and ovarian follicles for the onset and duration of estrus after a $\text{PGF}_2\alpha$ injection. It is hoped the

findings from this study will contribute towards a more complete understanding of the parameters which control estrous behavior as well as to provide insight into the causes of individual variation in the expression of estrus in the ewe.

MATERIALS AND METHODS

This study was conducted from September through December of 1982 at the Montana State Sheep Experiment Station located at Fort Ellis in Bozeman, Montana. Forty, normally cycling Western White-Faced ewes, aged six to seven years, were selected from a flock observed twice daily for standing estrus with the aid of 4 epididymectomized teaser rams. Three rams were run daily with the ewes, their briskets marked with grease paint. Marked ewes were separated from the flock and exposed to a fresh teaser ram to confirm standing estrus. Those ewes in heat were placed in a separate pen. Every 10 days the color grease paint was changed and the ewes in the "estrus pen" were returned to the flock. Only those ewes displaying at least 2 normal estrous cycles, 15 to 18 days in length, were included in this study.

Ewes and teaser rams were fed alfalfa grass hay, free-choice. Mineral supplements and water were also available.

Once the experimental flock had been selected, ewes were randomly assigned to 1 of 8 experimental groups (Table 1) to be either ovariectomized (OVX) or sham-operated (SO) at 36, 44, 52 or 60 h following a prostaglandin F_2^{α} injection (12.5 mg, I.M., Lutalyse; dinoprost tromethamine, 5mg / ml; Upjohn Co.) given on day 12 of the estrous cycle. This was designated as experimental hour zero (time 0h). At this time, and every 4 h afterwards extending for 96 h, each ewe was observed for standing estrus using a teaser ram.

At 36, 44, 52 or 60 h each ewe in that treatment group was OVX or SO via a mid-ventral laparotomy under thiameyl sodium (10 ml, 4%

Table 1. Experimental design including treatments and number of ewes per treatment.

Treatment	Time of surgery (h) ^a				Total
	36	44	52	60	
OVX	n=5	n=5	n=5	n=5	20
SO	n=5	n=5	n=5	n=5	20
Total	10	10	10	10	40

^aAfter PGF₂^α injection

Biotol; Bio-Ceutic Laboratories, Inc.; St. Joseph, MO) and halothane anesthesia (4% with .9 liters of oxygen per minute until a negative corneal response was observed, then 2 to 3% halothane with .6 liters of oxygen per minute during surgery; Hydrocarbon Laboratories, Inc.; Hackensack, NJ; Narxoyet Anesthesia stand model; semiclosed system with 4% halothane vaporizer) using aseptic techniques. Four hours prior to surgery, 12 ml of combiotic (200,000 IU per ml; Penicillin G Procaine V.S.P. in Dihydrostreptomycin Sulfate; Med-Tech Inc., Elwood, KS) was administered.

At the time of surgery all ovaries were examined macroscopically for follicular population. Follicles were counted, measured with a caliper and classified as small (1.0 to 3.9 mm), medium (4.0 to 5.9 mm) or large (6.0 mm or >). Ovaries that were removed were prepared for histological evaluation by first fixing the tissue in a 4 % buffered formalin solution for 24 h followed by dehydration in gradients of alcohol in distilled water for 6 h (30%), 10 h (50%) and storage (70%). Each ovary was cut in half and each half was embedded

in a paraffin block and labeled with ewe number, and half (A or B) of right or left ovary. Serial sections (10 microns) of tissue were taken using a microtome and sections were fixed onto slides, appropriately labeled with ewe information and tissue section number and stained with hematoxylin (Lerner-2 hematoxylin, Lerner Laboratories, New Haven, CT) and eosin (Eosin-Y, Lerner Laboratories, New Haven, CT).

Slides were evaluated and follicular diameter measured similar to the method developed by Rajakoski (1960). Slides were placed in serial order on a semi-transparent plastic sheet with a metric ruler included in the field. The sheet was illuminated from underneath and a photograph was taken. This process was repeated until all ovarian sections were included. Photographic slides were then projected onto a paper screen. Measurements of the CL and antral follicles were obtained by calibrating a hand held ruler to the ruler projected on the screen. Each follicle or CL was measured in millimeters at its widest vertical (d_1) and horizontal (d_2) point. A third measurement was taken by counting the number of sections in which a particular structure was observed. By multiplying that number by 10 microns, and dividing that value by 1,000 one obtained the measurement in millimeters (d_3). The average diameter was calculated by dividing $d_1 + d_2 + d_3$ by three. For CL, a volume measurement was taken as an estimate of CL weight. Volume was determined by dividing the average CL diameter by 2 to obtain the radius, cubing that number and multiplying it by the constant $4/3$ times pi. For ewes with more than

CL volume for an ovary.

Antral follicles were then classified histologically as either atretic or non-atretic. Criteria for atresia were: 1) the presence of a darkly stained and disassociated membrana granulosa, and 2) the appearance of atretic bodies. Non-atretic follicles maintained a bright uniform appearance and an intact membrana granulosa.

Corpora lutea were determined to be regressing if the luteal cells were misshapen and darkly stained, appeared to have an accumulation of lipid droplets indicating failure of steroid synthesis, and a breakdown in the luteal vascular system.

Statistical Analyses

Chi-square analysis was used to test for differences in the proportion of ewes in estrus after surgery (Snedecor and Cochran, 1980). Time to onset and duration of estrus, and estrous cycle length after PGF_2^α was analyzed using Harvey's least squares for unequal sample sizes (Harvey, 1979). One-way analysis of variance was used to test for differences in the cycle length prior to the onset of the experiment, and a paired t-test was used to determine differences in cycle length before and after the PGF_2^α injection for each time period (Snedecor and Cochran, 1980).

One-way analysis of variance with time as the classified variable was used to test ovarian populations for CL size, CL volume, total number of follicles, number of visible follicles, atretic follicles, non-atretic follicles, follicles on the right ovary, follicles on the left ovary, follicles on the CL ovary, follicles on the non-CL ovary, individual size classes of follicles

(small, medium and large), and condition of follicles (atretic or non-atretic follicles in each size class). Paired t-tests were used to test for differences in each time period for total number of visible and actual follicles, atretic and non-atretic follicles, follicles on the left and right ovary, follicles on the CL and non-CL ovary, comparison of follicle size classes, and the number of atretic and non-atretic follicles in each size class (Snedecor and Cochran, 1980). Level of significance for all tests was set at $P < 0.05$.

RESULTS

Comparison of Visible and Histological Methods in Assessing Ovarian Morphology

A common method used to examine ovarian activity in the ewe is to expose the ovaries via a midventral laparotomy and count and measure follicles and CL visible on the ovarian surface. This method is not always reliable because follicles and CL can be partially or completely buried within the stroma, sometimes making them difficult to see and measure with precision. On the other hand, there are problems associated with histological measurements due to tissue damage and shrinkage during preparation that make it difficult to accurately obtain absolute size measurements.

Results of this present study indicate both these problems exist. Ewes in the OVX group were used to examine the accuracy of this method. At the time of surgery, all visible antral follicles present on the surfaces of the ovaries were counted, a second count was taken during histological evaluation and included follicles concealed beneath the surface of the ovary. Comparison of the results of the two methods (Figure 1) indicated the actual number of antral follicles obtained histologically was greater than the number of visible follicles counted at 36 h ($P < .05$) and 60 h ($P < .05$). No differences were observed at 44 and 52 h ($P > .05$) which was a result of visible follicle counts being greater than actual histological counts for some ewes. This could be explained by misidentification of surface

follicles or duplicate counting of visible follicles while the ovaries were in situ.

The results demonstrate an accurate and precise count of the follicular population can not be obtained easily through visual appraisal of the ovary. The overall mean number of visible follicles was 29.6 ± 4.2 , and 57.0 ± 5.1 for actual follicles. In this study approximately 50% of the total follicles were not accounted for in the visual observation. The apparent low correlations within time between the two methods (Tables 9-12) weakens the possibility of predicting the actual number of follicles using the visible count. Based on these findings, the remaining analyses will involve only those ewes in the OVX group where an accurate follicle count could be obtained from histological evaluation.

Corpus luteum size and volume was determined both visually and microscopically in a manner similar to that for follicles. The reason for obtaining a volume measurement was that it has been shown that CL weight is most closely related to the level of progesterone synthesis (Plotka et al., 1970) and CL volume provides the best representation for CL weight in this study.

Table 7 illustrates that visible CL size ($7.3 \pm .5$ mm) did not differ ($P > .05$) from actual CL size ($6.8 \pm .4$ mm), nor did visible CL volume (367.2 ± 44.3 mm³) differ ($P > .05$, Table 8) from actual CL volume (355.5 ± 61.6 mm³) measurements. Although not significantly different, the visible measurements at times appear greater than the actual measurements. This probably reflects tissue shrinkage or damage that occurred during histological preparation.

It is apparent from these results that histological evaluation seems more advantageous than visible evaluation for obtaining a total count of structures, but is not necessarily more accurate in evaluating sizes.

Follicular Population Following $\text{PGF}_{2\alpha}$ Treatment

In OVX ewes the number of actual antral follicles remained constant and was not influenced by the time of surgery (Figure 1). These follicles were equally distributed ($P > .05$) between the left (28.4 ± 2.8) and right (28.7 ± 3.2) ovaries (Figure 2), and on the ovary bearing the CL (28.8 ± 3.6) and non-CL (29.6 ± 4.6) ovary ($P > .05$; Figure 3). Fogwell et al. (1977) reported that in the ewe, large follicles tended to grow around the CL, and similar findings were observed for the monkey and woman (Knobil, 1973). In this study there were no differences in the number of large follicles on the the CL and non-CL ovary ($P > .05$; Figure 4), and no differences in the number of large non-atretic follicles on the CL and non-CL ovary ($P > .05$; Figure 5).

The total follicular population was divided into small (1.0 to 3.9 mm), medium (4.0 to 5.9 mm) and large (> 6.0 mm) size classes and analysis indicated that for each size class the total number of follicles for each size class did not differ ($P > .05$) over time with 52.0 ± 11.6 small follicles, $3.6 \pm .5$ medium follicles and $1.4 \pm .2$ large follicles (Figure 6). Within each time period the number of small follicles was greater ($P < .05$) than the number of medium and large follicles. Also, the number of medium follicles was greater than the number of large follicles only at 36 h ($P < .05$).

Division of the follicular population into atretic and non-atretic populations showed that the average number of follicles in each group did not change over time ($P > .05$, Figure 7). However, Figure 7 seems to illustrate a decreasing trend, though not significant ($P > .05$), in the total number of non-atretic follicles from 36 to 52 h.

While the number of medium atretic follicles ($2.3 \pm .5$) did not change over time ($P > .05$), the population of medium non-atretic follicles fluctuated (Figure 8). The mean number of non-atretic follicles fell from $2.2 \pm .4$ follicles at 36 h to $1.0 \pm .4$ follicles at 44 h and $.2 \pm .2$ follicles by 52 h ($P < .05$). However, by 60 h the number of non-atretic follicles increased to $1.6 \pm .5$, which was greater than the number observed at 52 h ($P < .05$) and equivalent to that seen at 36 and 44 h ($P > .05$).

In the population of large follicles (Figure 9) the number of atretic follicles ($.5 \pm .2$) did not change over time ($P > .05$), while non-atretic follicles followed an biphasic pattern. The number of non-atretic follicles increased from a low of $.4 \pm .2$ follicles at 36 h, to a peak level of $1.8 \pm .8$ follicles at 52 h ($P < .05$), and by 60 h decreased to $.6 \pm .4$ follicles ($P < .05$).

Most of the estradiol produced originates from follicles 5 mm in size or greater (Baird, 1981; Moor, 1973), therefore large and medium follicles examined in this study were assumed to be the primary source of preovulatory estradiol and were considered as a pooled group of follicles (Figure 10). The number of atretic and non-atretic follicles did not differ over or within time ($P > .05$).

Size and Volume of Corpra Lutea Following PGF₂ α

Histologically, it was determined that all CL were actively undergoing regression. Visible CL size ($7.3 \pm .5$ mm) remained constant with time ($P > .05$;) as did visible CL volume (367.2 ± 44.3 mm³; $P > .05$). It did not appear that ewes with more than one CL had smaller individual CL sizes than ewes with only one CL. Results of actual CL size and volume are shown in Tables 7 and 8. Actual CL volume did not change over time ($P > .05$) and the overall mean volume was 355.5 ± 61.6 mm³, while CL size exhibited a gradual decline and by 60 hours CL size had shrunk to 5.0 ± 1.0 mm and was smaller than in all other time groups ($P < .05$). Although not significant, actual CL volume showed a decreasing trend with time. This may reflect regression of CL as the tissue shrinks and dies with time.

Length of the Estrous Cycle

On day 12 of the estrous cycle, all ewes were given a PGF₂ injection to induce luteal regression. To measure the effectiveness of PGF₂ α , the average estrous cycle length prior to the injection was compared to the cycle length following the injection for those ewes observed in estrus after PGF₂ α treatment (Table 2). The PGF₂ α injection shortened the estrous cycle to $13.7 \pm .2$ days from a previous cycle length of $17.1 \pm .2$ days ($P < .01$), indicating early luteal regression had taken place.

Effect of Treatment on the Estrous Response

The proportion of ewes exhibiting estrus was dependent on type of surgery (Table 3). In the ovariectomized (OVX) ewes 11 of 20 (55%) showed estrus compared to 20 of 20 (100%) of the sham-operated (SO)

ewes ($P < .05$). There was a tendency ($P < .10$) for a main effect of time of treatment on the proportion of ewes exhibiting estrus and a significant time by treatment interaction ($P < .05$) was observed. This indicated that type of surgery and the time it was performed influenced the number of ewes which came into estrus. Small sample sizes precluded further Chi-Square analysis to locate the source of the interaction. However, in the 36 and 52 h groups, 0 of 5 (0%) and 5 of 5 (100%) of OVX ewes, respectively, were observed in estrus, while 5 of 5 (100%) of SO ewes in the 36 and 52 h groups were in estrus.

The mean time to onset (TO) of estrus following $\text{PGF}_2\alpha$ was 48.3 ± 3.6 h for OVX ewes that showed estrus and 52.2 ± 2.4 h for SO ewes (Table 4). No time or treatment effects were observed ($P > .05$), and the overall mean was 50.7 ± 1.9 h. This result falls within the range of 44 to 66 h reported by Chamely et al. (1976).

Duration of estrus (Table 5) was 25.6 ± 4.8 h in OVX ewes that showed estrus and 32.6 ± 3.2 h for SO ewes. No time or type of surgery effects were apparent ($P > .05$), and the overall mean duration of estrus was 29.8 ± 2.5 h, which lies within the range of 24 to 48 h reported by Hafez (1974).

Effect of Ovarian Morphology on Behavioral Estrus

Pearson correlations were used to evaluate the effect of CL and follicular populations on TO and duration of estrus (Table 6). Only large and medium follicles and their non-atretic counterparts were considered in the follicular population since it is primarily these follicles which produce the estrogen needed to induce behavioral estrus. It should be noted that since not all ewes were observed in

estrus because of OVX, their data were eliminated from the analyses. Also, in this study, only ewes that were OVX and had an accurate histological evaluation of the follicular population were considered (see comparison of visible and histological analysis in measurements of ovarian morphology on page 35). This resulted in reduced sample sizes and these correlation analyses must be interpreted with caution. Therefore, the objective of this analysis was to look for possible relationships rather than draw firm conclusions.

The relationship of total number of follicles to TO and duration of estrus appears low, as well as inconsistent over time, showing both positive and negative correlations (Table 6). Similar inconsistencies over time are apparent for the relationship of number of non-atretic, medium, medium non-atretic, large and medium, and large and medium non-atretic follicles. In general, the above mentioned structures appear to be negatively associated with time to onset of estrus and positively associated with duration of estrus.

The strongest relationships became apparent when the population of large follicles was analyzed. Within time of surgery correlations were high and positive for the duration of estrus with $r=.76$ (44 h), $r=.66$ (52 h) and $r=.76$ (60 h), and negative for TO of estrus at 44 ($r= -.97$), 52 ($r= -.67$) and 60 h ($r= -.50$). For large non-atretic follicles the relationships were even larger with negative correlations for TO of estrus at 44 ($r= -.97$), 52 ($r= -.73$) and 60 h ($r= -.87$), and positive correlations for duration of estrus at 44 ($r=.76$), 52 ($r=.70$) and 60 h ($r=.65$).

Both visible and actual measurements of CL were included in the correlation analysis (Table 6) since no differences were found using the two measurement techniques (see comparison of visible and histological methods in assessing ovarian morphology on page 35). Correlations for visible CL size and volume on the TO of estrus were very low but tended to be positive, with the only significant correlation ($P < .05$) for CL volume at 36 h ($r = .90$). For duration of estrus, the correlation coefficients were generally low and positive for both measurements, with a significant correlation ($P < .05$) at 36 h ($r = .68$) for CL volume. The correlation of actual CL size was a mix of both positive and negative values for the TO and duration of estrus. Of interest was the consistent relationship shown by actual CL volume. For duration of estrus there was a positive correlation at 44 ($r = .84$), 52 ($r = .58$) and 60 h ($r = .68$), and a negative correlation for TO of estrus 44 ($r = -.93$), 52 ($r = -.36$) and 60 h ($r = -.39$).

Table 2. Estrous cycle length before and after PGF_2^α injection.^a

Before PGF_2^α	After PGF_2^α
$17.4 \pm .2^b$	$13.7 \pm .2^c$

^a $\bar{X} \pm \text{SEM}$ ^{b,c}Different superscripts in same row indicate differences at $P < .05$ Table 3. Proportion (%) of PGF_2^α -treated ewes showing estrus after surgery.

Treatment	Time of surgery (h) ^a				Total
	36	44	52	60	
OVX	0/5	3/5	5/5	3/5	11/20 (55.5%) ^b
SO	5/5	5/5	5/5	5/5	20/20 (100%) ^c
Total	5/10 ^c (50%)	8/10 ^{bc} (80%)	10/10 ^b (100%)	8/10 ^{bc} (80%)	31/40 (77.5%)

^aType of surgery X time interaction, $P < .05$ ^{b,c}Different superscripts in column totals indicate differences at $P < .05$ and in row totals at $P < .10$

Table 4. Effect of treatment on time to onset (h) of estrus after a $\text{PGF}_2\alpha$ injection in the ewe.^a

Treatment	Time of surgery (h)				Total
	36	44	52	60	
OVX	-----	46.7 $\pm$ 3.5	44.0 $\pm$ 2.2	56.0 $\pm$ 7.1	48.3 $\pm$ 3.6
SO	50.4 $\pm$ 2.8	52.8 $\pm$ 4.1	49.6 $\pm$ 1.8	56.0 $\pm$ 6.2	52.2 $\pm$ 2.4
					50.7 $\pm$ 1.9 ^b

^a $\bar{X} \pm \text{SEM}$ ^bOverall $\bar{X} \pm \text{SEM}$ Table 5. Effect of treatment on duration (h) of estrus in $\text{PGF}_2\alpha$ -treated ewes.^a

Treatment	Time of surgery (h)				Total
	36	44	52	60	
OVX	-----	17.3 $\pm$ 3.6	27.2 $\pm$ 4.5	28.0 $\pm$ 12.5	25.6 $\pm$ 4.8
SO	36.8 $\pm$ 6.0	32.0 $\pm$ 6.7	32.0 $\pm$ 7.0	29.6 $\pm$ 7.0	32.6 $\pm$ 3.2
					29.8 $\pm$ 2.5 ^b

^a $\bar{X} \pm \text{SEM}$ ^bOverall $\bar{X} \pm \text{SEM}$

Table 6. Correlation coefficients for corpora lutea(CL) and follicular morphology on time to onset and duration of estrus.

Variable ^a	Time of surgery (h)									
	36 ^b	44	52	60	T	36	44	52	60	T
	Time to onset					Duration				
T	—	.35	-.52	-.27	.16	—	.56	.27	-.06	.05
NA	—	.38	-.32	.31	.21	—	.54	-.27	-.60	-.29
L	—	-.97 ^C	-.67	-.50	-.46	—	.76	.66	.76	.66 ^C
LNA	—	-.97 ^C	-.73	-.87	-.84 ^C	—	.76	.70	.65	.36
M	—	.83	-.63	.87	.58 ^C	—	-.93 ^C	.78 ^C	-.65	-.16
MNA	—	-.97 ^C	-.46	.65	.41	—	.76	.49	-.37	-.20
LMNA	—	-.97 ^C	-.67	.50	-.32	—	.76	.66	-.19	.11
LM	—	.28	-.66	.50	.20	—	-.94 ^C	.73	-.19	.23
CLsa	—	-.93 ^C	-.55	.65	-.30	—	.84	.39	-.37	-.28
CLva	—	-.93 ^C	-.36	-.39	-.19	—	.84	.58	.68	.56 ^C
CLsv	-.33	.08	.27	.36	.16	-.54	.00	.36	.10	.25
CLvv	.90 ^C	.04	.44	-.09	.17	.68 ^C	.37	.15	.08	.41

^a(T) total number of follicles, (NA) non-atretic, (L) large, (LNA) large non-atretic, (M) medium, (MNA) medium non-atretic, (LMNA) large and medium non-atretic, (LM) large and medium, (CLsa) actual CL size, (CLva) actual CL volume, (CLsv) visible CL size, (CLvv) visible CL volume

^b36 h data are not shown since ewes in these groups did not show estrus, except for visible CL measurements that included sham groups in which ewes were in estrus at 36 h.

^cP<.05

Table 7. Means for visible and actual corpora lutea (CL) sizes (mm^3) in ovariectomized ewes.^a

Measurement	Time of surgery (h)				Total
	36	44	52	60	
Visible	7.3 ± 1.2	7.8 ± 1.0	$7.3 \pm .4$	6.8 ± 1.3	$7.3 \pm .5$
Actual	$7.8 \pm .4^b$	$7.4 \pm .3^b$	$7.0 \pm .8^b$	5.0 ± 1.0^c	$6.8 \pm .4$

^aMeans in same column did not differ ($P > .05$)

^{b,c}Means in same row with different superscripts indicate differences at $P < .05$

Table 8. Means for visible and actual corpora lutea (CL) volumes (mm^3).^a

Measurement	Time of surgery (h)				Total
	36	44	52	60	
Visible	435.3 ± 81.3	376.0 ± 101.6	282.0 ± 73.0	375.7 ± 96.8	367.2 ± 44.3
Actual	434.4 ± 108.4	378.7 ± 126.1	365.4 ± 166.6	243.8 ± 122.8	355.5 ± 61.6

^aMeans in same rows and columns did not differ ($P > .05$)

Table 9. Correlation coefficients for relationships in ovarian morphology at 36 hours following a PGF₂ injection.^a

	Sac	Mac	Lac	IMA	MA	LA	CLsv	CLvv	CLsa	V	A	RO	FCL	LCL	LNACL	LNARO
Mac	-.60															
Lac	.84	.00														
LMNA				-.89												
MNA					-.84											
LNA						-.67										
CLvv							.61									
CLsa							.35									
CLva								.19	.85							
Sv	.37															
Mv		.20														
Lv			-.88													
Ac										.24						
NA										-.06						
EO											.68					
FNCL												.94				
LNCL													-.58			
LNANCL														.00		
LNALO																.00

^a(Sac) number of small actual follicles, (Mac) medium actual follicles, (Lac) large actual follicles (IMA) large and medium atretic follicles, (LMNA) large and medium non-atretic follicles, (MA) medium atretic follicles, (MNA) medium non-atretic follicles, (LA) large atretic follicles, (LNA) large non-atretic follicles, (CLsv) visible CL size, (CLvv) visible CL volume, (CLsa) actual CL size, (CLva) actual CL volume, (V) visible follicles, (Ac) actual follicles, (A) atretic follicles, (NA) non-atretic follicles, (FCL) follicles on CL ovary, (FNCL) follicles on non-CL ovary, (RO) follicles on right ovary, (EO) follicles on left ovary, (FCL) large follicles on CL ovary, (LNCL) large follicles on non-CL ovary, (LNACL) large non-atretic follicles on CL ovary, (LNANCL) large non-atretic follicles on non-CL ovary, (LNARO) large non-atretic follicles on right ovary, (LNALO) large non-atretic follicles on left ovary.

Table 10. Correlation coefficients for relationships in ovarian morphology at 44 hours following a PGF₂ injection.^a

	Sac	Mac	Lac	IMA	MA	LA	CLsv	CLvv	CLsa	V	A	RO	FCL	LCL	LNACL	LNARO
Mac	.06															
Lac	-.02	.00														
IMNA				-.64												
MNA					-.72											
LNA						-.46										
CLvv							.44									
CLsa							-.25									
CLva								.30	-.22							
Sv	.58															
Mv		-.09														
Lv			-.61													
Ac										.48						
NA											.92					
LO												.72				
FNCL													.89			
LNCL														.00		
LNACL															.00	
LCALO																-.10

^a(Sac) number of small actual follicles, (Mac) medium actual follicles, (Lac) large actual follicles (IMA) large and medium atretic follicles, (IMNA) large and medium non-atretic follicles, (MA) medium atretic follicles, (MNA) medium non-atretic follicles, (LA) large atretic follicles, (LNA) large non-atretic follicles, (CLsv) visible CL size, (CLvv) visible CL volume, (CLsa) actual CL size, (CLva) actual CL volume, (V) visible follicles, (Ac) actual follicles, (A) atretic follicles, (NA) non-atretic follicles, (FCL) follicles on CL ovary, (FNCL) follicles on non-CL ovary, (RO) follicles on right ovary, (LO) follicles on left ovary, (LCL) large follicles on CL ovary, (LNCL) large follicles on non-CL ovary, (LNACL) large non-atretic follicles on CL ovary, (LNANCL) large non-atretic follicles on non-CL ovary, (LNARO) large non-atretic follicles on right ovary, (LNALO) large non-atretic follicles on left ovary.

Table 11. Correlation coefficients for relationships in ovarian morphology at 52 hours following a PGF₂ injection.^a

	Sac	Mac	Lac	IMA	MA	LA	CLsv	CLvv	CLsa	V	A	RO	FCL	LCL	LNACL	LNARO
Mac	.44															
Lac	.72	.94														
IMNA				.94												
MNA					.56											
LNA						.80										
CLvv							-.14									
CLsa							.95									
CLva								.32	.92							
Sv	-.15															
Mv		-.02														
Lv			.46													
Ac										-.19						
NA										-.22						
LO												.10				
FNCL													-.10			
LNCL														.00		
LNANCL															.00	
LNALO																.00

^a(Sac) number of small actual follicles, (Mac) medium actual follicles, (Lac) large actual follicles (IMA) large and medium atretic follicles, (IMNA) large and medium non-atretic follicles, (MA) medium atretic follicles, (MNA) medium non-atretic follicles, (LA) large atretic follicles, (LNA) large non-atretic follicles, (CLsv) visible CL size, (CLvv) visible CL volume, (CLsa) actual CL size, (CLva) actual CL volume, (FCL) follicles on CL ovary, (FNCL) follicles on non-CL ovary, (RO) follicles on right ovary, (LO) follicles on left ovary, (LCL) large follicles on CL ovary, (LNCL) large follicles on non-CL ovary, (LNACL) large non-atretic follicles on CL ovary, (LNANCL) large non-atretic follicles on non-CL ovary, (LNARO) large non-atretic follicles on right ovary (LNALO) large non-atretic follicles on left ovary.

Table 12. Correlation coefficients for relationships in ovarian morphology at 60 hours following a PGF₂ injection.^a

	Sac	Mac	Lac	IMA	MA	LA	CLsv	CLvv	CLsa	V	A	RO	FCL	LCL	LNACL	LNARO
Mac	-.55															
Lac	-.31	.38														
IMNA				.58												
MNA					.23											
LNA						-.74										
CLvv							.37									
CLsa							.04									
CLva								.80	.77							
Sv	-.05															
Mv		.53														
Lv			.41													
Ac										-.33						
NA											-.37					
LO												-.10				
FNCL													.12			
LNCL														-.82		
LNANCL															-.58	
LNALO																-.41

^a(Sac) number of small actual follicles, (Mac) medium actual follicles, (Lac) large actual follicles (IMA) large and medium atretic follicles, (IMNA) large and medium non-atretic follicles, (MA) medium atretic follicles, (MNA) medium non-atretic follicles, (LA) large atretic follicles, (LNA) large non atretic follicles, (CLsv) visible CL size, (CLvv) visible CL volume, (CLsa) actual CL size, (CLva) actual CL volume, (V) visible follicles, (Ac) actual follicles, (A) atretic follicles, (NA) non-atretic follicles, (FCL) follicles on CL ovary, (FNCL) follicles on non-CL ovary, (RO) follicles on right ovary, (LO) follicles on left ovary, (LCL) large follicles on CL ovary, (LNCL) large follicles on non-CL ovary, (LNACL) large non-atretic follicles on CL ovary, (LNANCL) large non-atretic follicles on non-CL ovary, (LNARO) large non-atretic follicles on right ovary, (LNALO) large non-atretic follicles on left ovary.

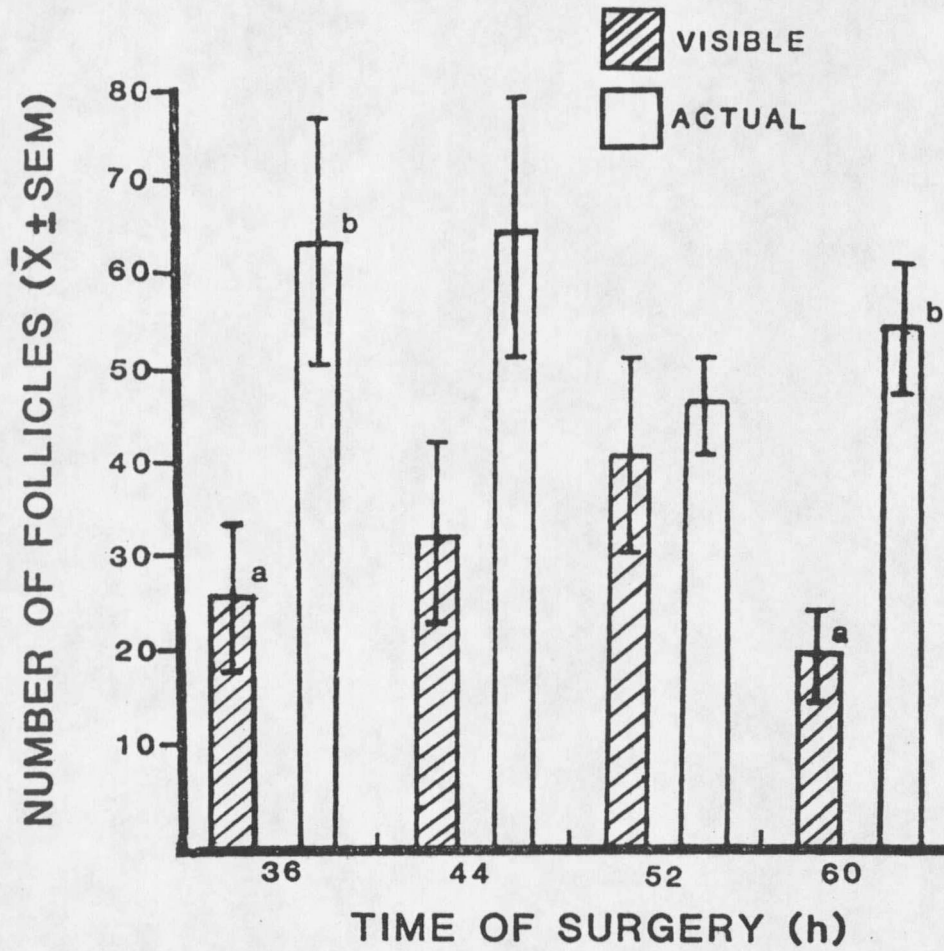


Figure 1. Mean number of visible follicles and actual follicles present on the ovaries at the time of surgery.

^{a,b} Different superscripts indicate differences ($P < 0.05$) within each time group.

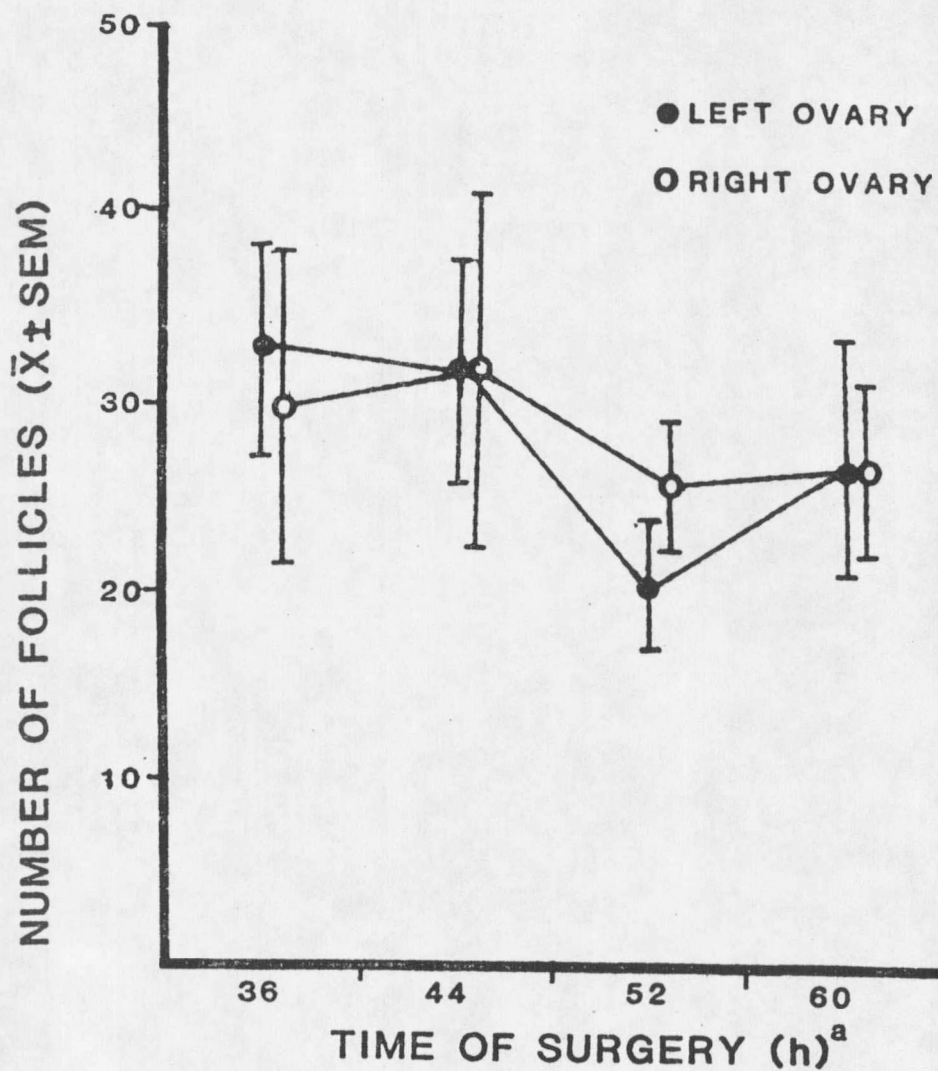


Figure 2. Mean number of total actual follicles present on the left and right ovaries at the time of surgery.

^aNo differences within each time group, or among the left or right ovary with time ($P > .05$).

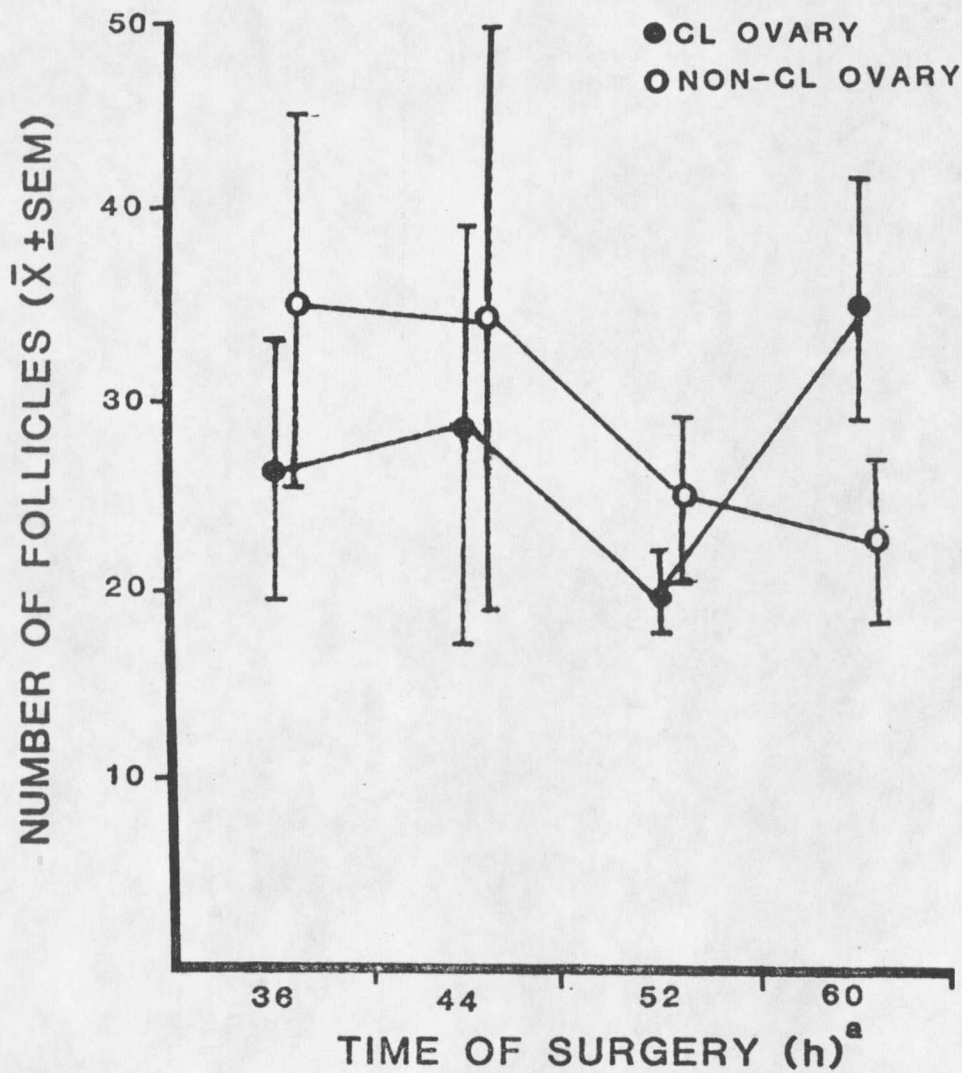


Figure 3. Mean number of total actual follicles on the CL and non-CL ovary at the time of surgery.

^aNo time differences were observed for follicles on the CL or non-CL ovary ($P > .05$) or between the CL and non-CL ovary within each time group ($P > .05$).

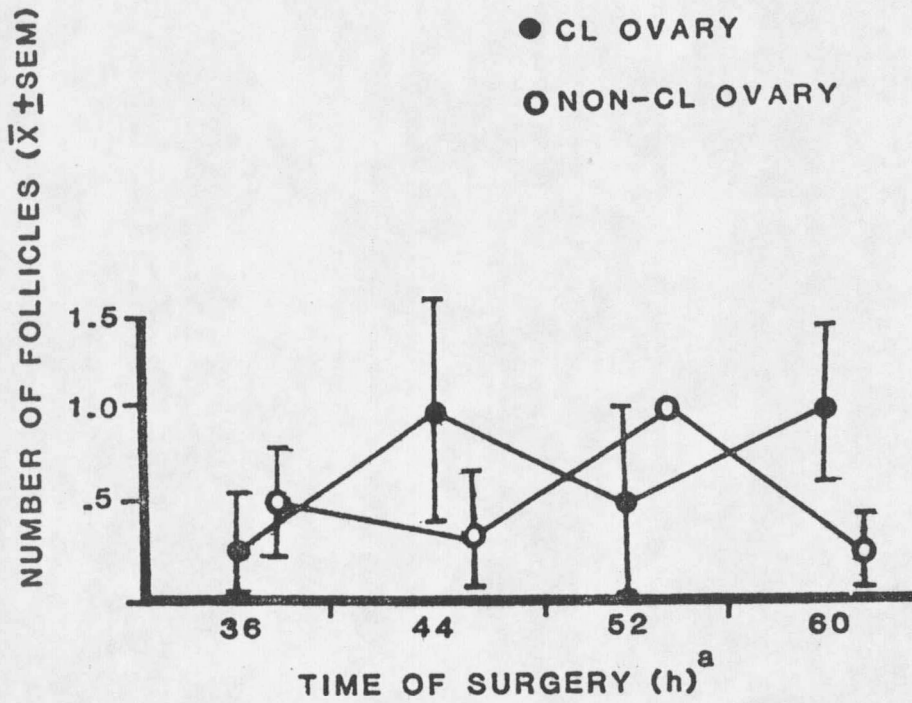


Figure 4. Mean number of actual large follicles present on the CL and non-CL ovary at the time of surgery.

^aNo time differences were observed for follicles on the CL or non-CL ovary or between the CL and non-CL ovary within each time ($P > .05$).

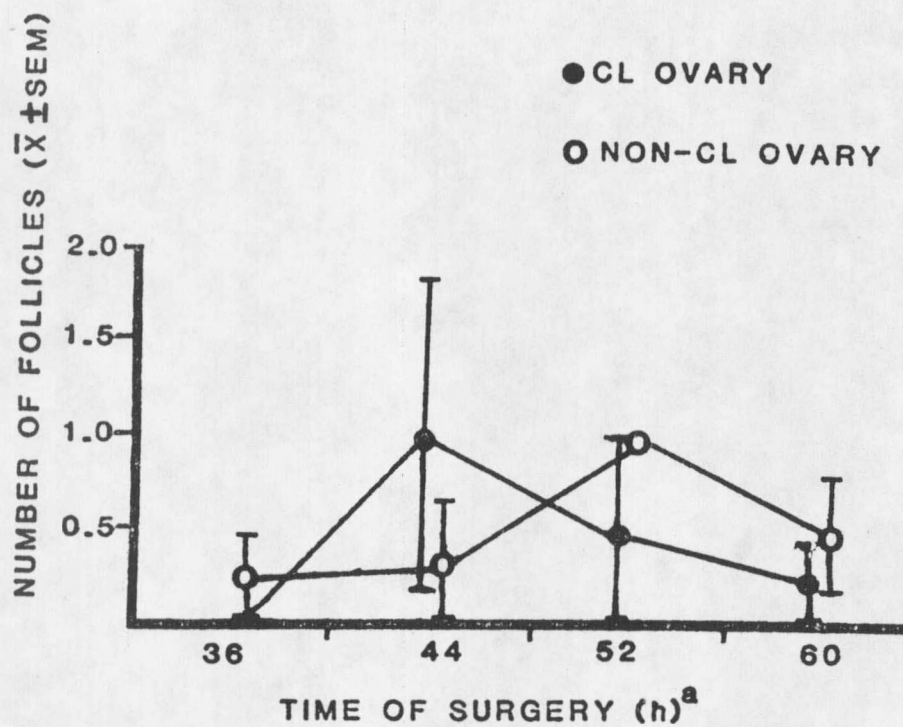


Figure 5. Mean number of actual large non-atretic follicles present on the CL and non-CL ovary at the time of surgery.

^aNo differences were observed for follicles on the CL or non-CL ovary ($P > .05$), or between the CL and non-CL ovary within each time ($P > .05$).

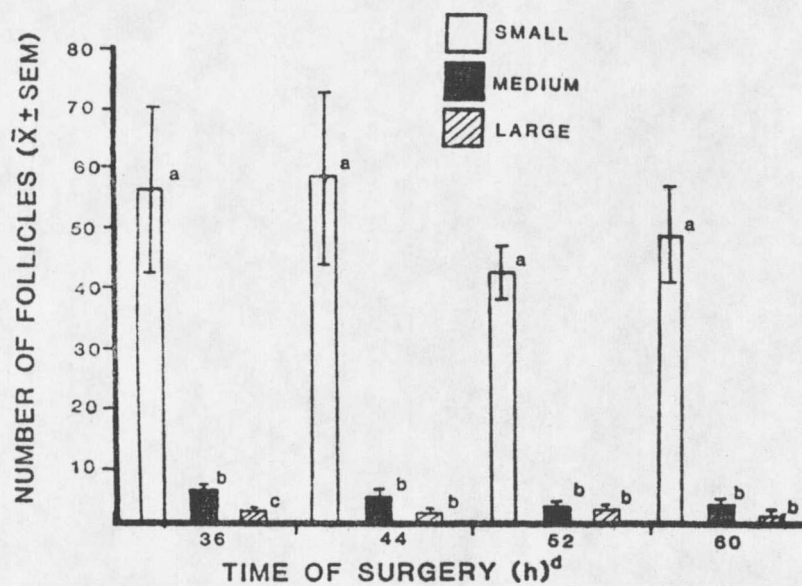


Figure 6. Mean number of actual small, medium and large follicles on the ovaries at the time of surgery.

a,b,c Different superscripts indicate differences within each time group ($P < .05$).

^dNo time differences were observed for the number of small, medium or large follicles ($P > .05$).

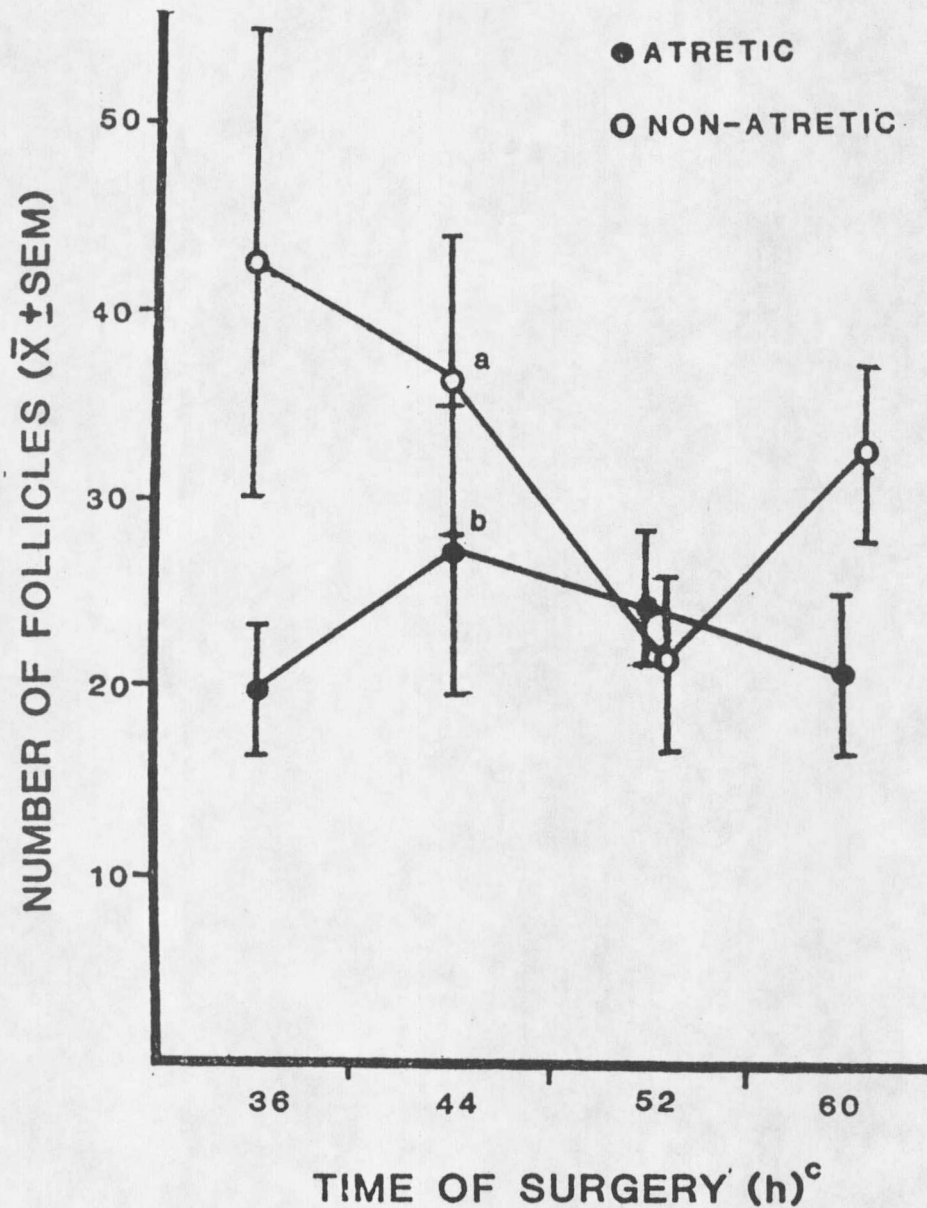


Figure 7. Mean number of actual atretic and non-atretic follicles present on the ovaries at the time of surgery.

^{a,b} Different superscripts indicate differences within each time group for atretic and non-atretic follicles ($P < .05$).

^c No time differences were observed for the number of atretic or non-atretic follicles ($P > .05$).

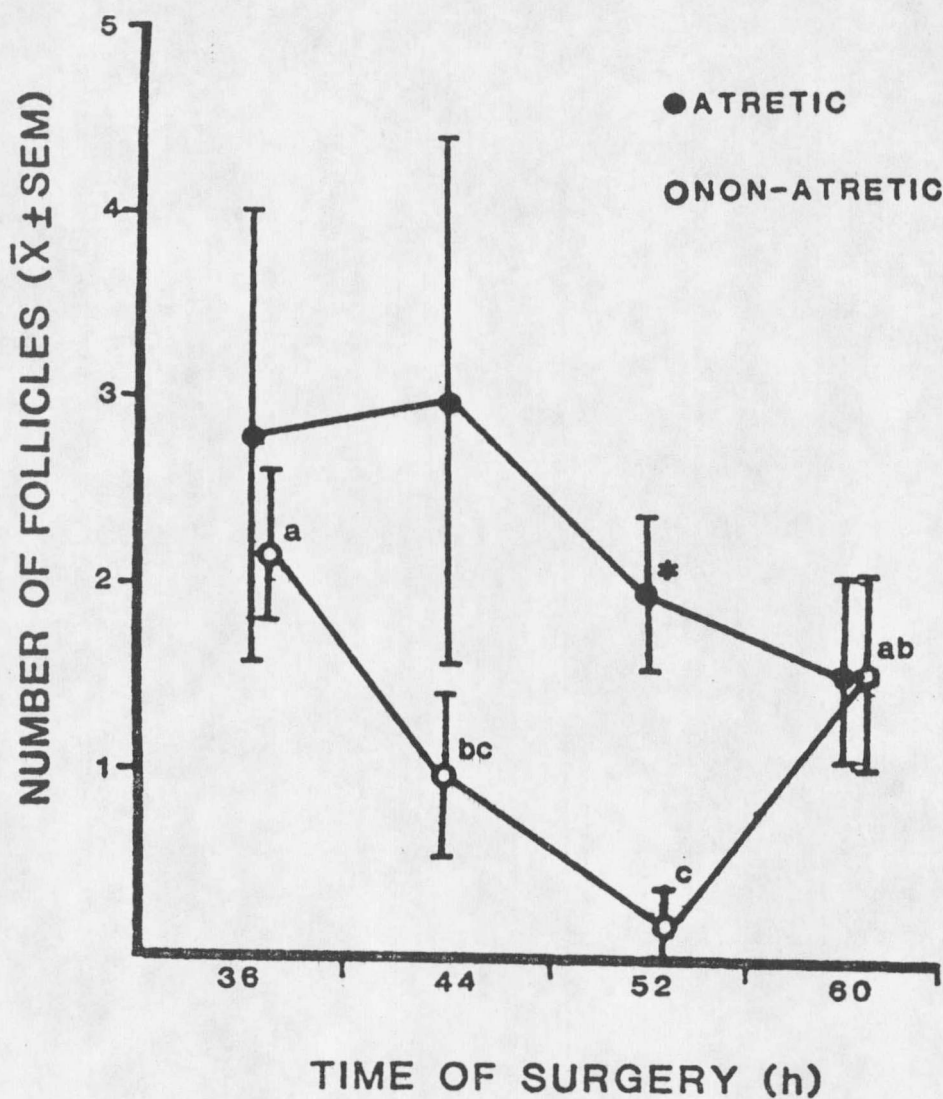


Figure 8. Mean number of actual medium atretic and non-atretic follicles on the ovaries at the time of surgery.

a,b,c Different superscripts indicate differences at these times for non-atretic follicles ($P < .05$).

* Indicates the number of atretic follicles was greater than the number of non-atretic follicles in that time group ($P < .05$).

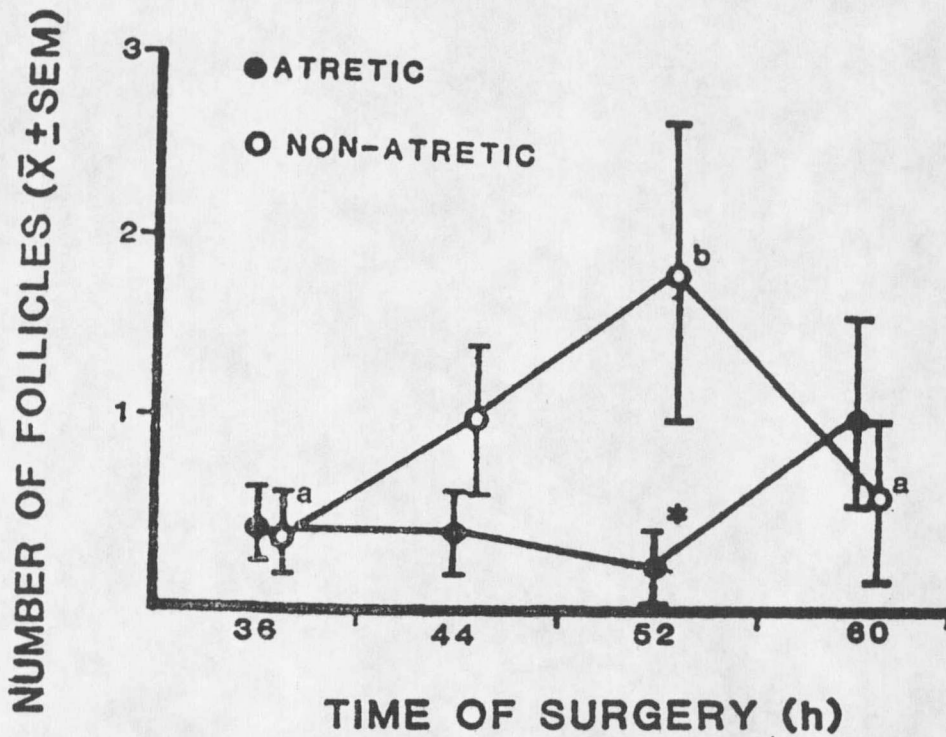


Figure 9. Mean number of actual large atretic and non-atretic follicles on the ovaries at the time of surgery.

a,b Different superscripts indicate differences at these times for non-atretic follicles ($P < .05$).

* Indicates the number of non-atretic follicles was greater than the number of atretic follicles in that time group ($P < .05$).

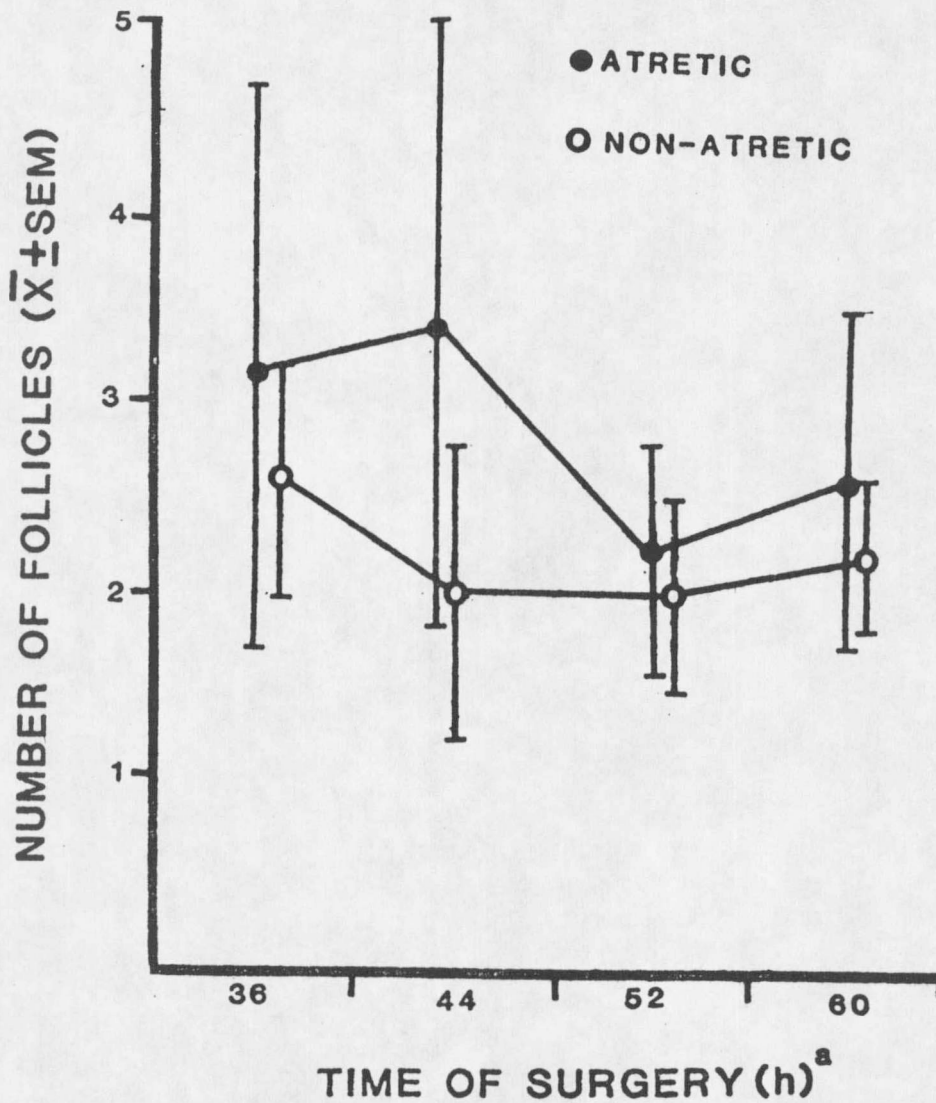


Figure 10. Mean number of actual large and medium atretic and non-atretic follicles present on the ovaries at the time of surgery.

^aNo time differences were observed for atretic or non-atretic follicles, or between atretic and non-atretic follicles within each time ($P > .05$).

DISCUSSION

It is evident from the results of this study that a temporal relationship exists between the ovary and the occurrence of behavioral estrus. It has been established that estrous behavior in the ewe occurs after preovulatory surge levels of estradiol begin to decline (Baird, 1978; Shemesh et al., 1972; Short, 1972) and that the full preovulatory increase in estradiol is not essential for estrous behavior to occur (Goodman, 1978). This has led to the belief that once estrogen reaches a "critical" level, estrous behavior is triggered and further estrogen secretion is not required for the normal duration of estrus. Also, since estrus is observed after the decline in estrogen levels, there may be a latent period from the time the "critical" level is reached and the time behavioral estrus is initiated. There is evidence in the rat (Johnston and Davidson, 1979) for a minimum period of time (30 h) in which they must be exposed to estrogen to induce normal lordosis. A similar condition may exist for the ewe.

This idea is supported in part by this study. Duration of estrus did not differ between those ewes observed in estrus for the OVX and SO ewes, indicating that once estrus behavior was initiated, the continuous presence of the ovary was not required for the normal duration of estrus. Although the duration of estrus did not differ between the two surgery groups, it tended to be shorter in OVX ewes. This may relate to the length of time the ewes were exposed to elevated levels of estrogen, in that ewes that were OVX had limited

exposure and may not have met the "minimum" requirement for duration of estrogen stimulation and were therefore in estrus for a shorter period of time.

The exact time requirement of the ovary following $\text{PGF}_2\alpha$ for the onset of estrus could not be determined from this study. If indeed there is a latent period between the "critical" level of estradiol and the onset of estrus, one would expect the ovaries could be removed prior to the onset of estrus and still have estrus occur. Two of 11 ewes in the OVX group which came into estrus, did so after surgery, indicating the possibility that a latent period does exist. Furthermore, it appears that the presence of the ovary, or more specifically, preovulatory follicles, are required for at least some time between 36 and 44 h, but probably longer, to produce the amount of estrogen and (or) other factors required to trigger behavioral estrus. This may explain the failure of some OVX ewes to come into estrus in the 36, 44 and 60 h groups. The average time span to the onset of estrus after a $\text{PGF}_2\alpha$ injection is 44 to 66 h (Chamley et al., 1972) and this may relate to individual variation in the rate of decline of progesterone secretion and subsequent rise in estradiol. Those ewes not observed in estrus may have been ovariectomized (OVX) before estradiol reached the peak level required to induce behavioral estrus. This "critical" level probably occurs after 36 h since no ewes were observed in estrus when OVX at this time.

The fact that both estrogen (Anderson, 1969) and progesterone (Robinson, 1954) act to hasten the onset of estrus lend support to this idea. Results of this study indicate the number of large non-

atretic follicles and actual CL volume are negatively associated with time to onset of estrus. Actual CL volume was the best approximation available in this analysis for CL weight, which has been determined to be closely related to progesterone output. It is possible that ewes with larger CL volumes (or weights) prior to regression may produce more progesterone and prime the central nervous system to respond to lower "critical" levels of estradiol, and ewes with a greater number of large non-atretic follicles may produce higher amounts of estradiol and the estradiol levels may peak sooner. Both of the above conditions could hasten the onset of estrus.

Duration of estrus in ewes can range from 24 to 48 h (Hafez, 1974). Land et al. (1972) demonstrated with progesterone-treated OVX ewes that duration of estrus was related to dosage of exogenous estradiol and could be prolonged with higher doses of estradiol given over an extended period of time. Therefore, variations in intact ewes may be due to individual differences in the amount of estradiol produced during the preovulatory period prior to the onset of estrus. Levels of estradiol were not measured in this study, however some correlations suggested a positive relationship between the number of large non-atretic follicles present on the ovaries and the duration of estrus. If ewes with more large non-atretic follicles are capable of producing more estradiol, thus increasing the duration of estrus, the positive association could be explained.

Previous studies on the patterns of follicular growth indicate that in the ewe, the total number of follicles does not change with day of the estrous cycle. However, follicular diameter tends to

increase towards the end of the estrous cycle (Kammlade et al., 1952). The same pattern of rapid follicular growth and replacement of large follicles at the end of the cycle is evident in cattle (Matton et al., 1981). Results of this study were in agreement and may suggest a narrowing of the population of preovulatory follicles whereby those follicles not destined to ovulate (i.e. growth stage of follicle out of phase with preovulatory events) drop out of the non-atretic pool.

Examination of individual size classes of follicles lend support to this hypothesis. While the total number of follicles remained constant, there was a shift in class (atretic and non-atretic) of large and medium follicles. From 36 to 52 h the number of medium non-atretic follicles decreased, while the number of large non-atretic follicles increased. This change could be explained by the movement of medium non-atretic follicles over time into the large size class during a period of rapid follicular growth in response to preovulatory hormone changes. The fact that the number of medium atretic follicles at 52 h ($2.0 \pm .4$) is greater than the corresponding number of non-atretic follicles may indicate some of these follicles are lost to atresia as preovulatory endocrine processes eliminate portions of the population. The increase in medium non-atretic follicles by 60 h may represent a "recruitment" of new follicles for the next preovulatory period, one or some of which may be the next ovulatory follicle at the subsequent estrus.

The increase in the number of large non-atretic follicles from 36 to 52 h can be explained using the same hypothesis. As small and medium non-atretic follicles grow into the next size class or become

atretic, a decline in their numbers was seen, while at the same time the number of large non-atretic follicles increased as smaller follicles became large follicles and fewer large follicles became atretic. The decline in the number of large non-atretic follicles by 60 h may indicate a "decision" had been made in the selection of the preovulatory follicle(s) and the remaining follicles began to die. The total number of follicles in each size class remain constant as enough follicles enter the growth phase to become small follicles, small follicles become medium follicles, and enough medium and large follicles become atretic to maintain the population balance (Brand and Jong, 1973).

The results of these follicular analyses suggest that during the period immediately following the $\text{PGF}_2\alpha$ injection, follicles undergo a period of rapid growth and degeneration, whereby follicles either grow into the next size class or become atretic in response to preovulatory hormone changes. It appears from this study that a critical turnover point in the follicular population occurs at 52 h, at which time small and medium follicles either continue to grow or begin to die. Perhaps large follicles are better able to respond to preovulatory hormone changes and remain in the non-atretic state. If the original pool of large non-atretic follicles can remain constant, then the population of smaller follicles growing into the large size class can allow for the increasing trend over a 52 h period. Sometime around 52 h, possibly, a "choice" had been made as to which follicle(s) will ovulate and the decline in non-atretic follicles 4 h later may suggest the remainder of the follicles not chosen began to die off.

The factors that determine which follicles will continue to grow and ovulate are many. Only those follicles in an active period of growth at the time of luteal regression will eventually be able to ovulate (Smeaton and Robinson, 1971). These follicles must develop an adequate number of LH receptors in both theca and granulosa layers to be capable of responding to the LH surge with ovulation (Webb and England, 1982). Atresia may result for those follicles unable to respond to preovulatory increases in gonadotropins (Thibault et al., 1975). Large non-atretic follicles are preserved by their ability to produce elevated estrogen levels which are antiatretic (Harman et al., 1975) and their decreased dependence on gonadotropins for continued growth. The preovulatory rise in gonadotropins can cause follicles whose growth stage is "out of step" with preovulatory events to become atretic. This may be attributed to a low number of LH receptors, insufficient sensitivity to LH, or development of the mechanism to respond to LH surge too late or too soon with respect to the LH surge.

To more clearly define the role of preovulatory follicles on the time to onset and duration of estrus, this experiment could be repeated with a modified experimental design. Sample sizes should be increased to at least 10 ewes per treatment group for statistical purposes. Apparently, the presence of the ovary is required until some time after 36 h following $\text{PGF}_2\alpha$; therefore, surgical schedules should be changed to begin at 40 h and extend out to 70 h to encompass the variation of 44 to 66 h until the onset of estrus. This would also give a better picture of follicular changes that occur during a

short period of time. Once again, every 4 h the ewes should be checked for standing estrus for a 96 h period. With each estrus check, a blood sample should be taken and evaluated for progesterone, estradiol and luteinizing hormone to relate changes in preovulatory hormone levels to the time to onset and duration of estrus.

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