SOME TEMPORAL RELATIONSHIPS BETWEEN THE ANTERIOR PITUITARY, CORPORA LUTEA AND ENDOMETRIUM OF NULLIPAROUS GILTS DURING THE ESTROUS CYCLE

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ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. M. C. Morrissette, Professor of Physiology and Pharmacology, for his interest, and guidance during the course of this study, and in the preparation of this thesis.

Appreciation is also extended to Mrs. Hsin Hsin Yang and to Mr. Whitney Williams for their able technical assistance; to Dr. Robert D. Morrison for statistical computations; to Dr. Roger Panciera for photographic assistance; and to the Endocrinology Study Section for supplies of NIH-FSH-P_1, NIH-FSH-S_1, NIH-LH-B_4, and NIH-LH-S_{11}.

The author also wishes to acknowledge the financial assistance of the National Institute of Child Health and Human Development, USPHS, through Postdoctoral Fellowship, 5F2 HD-30, 234-03 and Research Project Grant HD-01088 and the Oklahoma Agricultural Experiment Station Project 1081.

The author wishes above all to acknowledge appreciation to his wife and children for their patience and many sacrifices necessary over the years in preparing the foundation for this work.

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CHAPTER I

INTRODUCTION

An important aspect of reproductive physiology is the development, maintenance and control of the corpus luteum during the estrous cycle. The basis for a unifying concept to perfect estrus cycle control and synchronization depends on a fuller knowledge of estrous cycle physiology for each individual species, though comparative studies should be fruitful.

The sequence and interaction of events which promote development, function, maintenance and regression of luteal tissue will be understood only after elucidation of the temporal relationships for the following parameters during the estrous cycle; (a) the concentration of tissue and plasma gonadotropins and gonadal steroids; (b) the nature and role of luteotrophic and luteolytic mechanisms; (c) the endometrial and ovarian enzyme changes; and (d) the morphological changes in ovarian and uterine structures. Recently some progress has been made because new techniques needed to evaluate certain of these parameters have evolved. Much more remains to be done.

Early investigators have characterized the estrous cycle from superficial observations and from certain facts about the estrous cycle such as length, time of ovulation and certain gross as well as microscopic ovarian and uterine changes.

The information available on the regulation of hypothalamic centers, gonadotropin and gonadal steroid secretion and endocrine effects on

target tissues have come from the fragmentary evidence accumulated by numerous investigators working with different species. However, little effort to relate these factors temporally, day by day, in a precise manner in any one species has been made. The lack of knowledge concerning control of the periodicity in the female cycle has been due, in part to the absence of precise and sensitive methods for measuring blood levels of the gonadotropic and gonadal hormones. Recently better methods have been developed.

More recent investigations have shown that pituitary luteotropic and/or endometrial mechanisms are concerned with the direct control of luteal functions. Much confusion exists as to the identity of these factors and their mode of action and definite contradictions between species have been shown to exist. This in turn has emphasized the necessity for comparative investigations into the temporal mechanisms of estrous cycle regulation.

In view of the considerations just stated a four-part experiment was designed to determine, within a group of cycling nulliparous swine, the temporal relationships between ovarian morphologic changes, pituitary and plasma FSH and LH concentrations, luteal progesterone concentrations and endometrial and luteal phosphatase activities for each day of the cycle. This study should provide a better understanding of estrous cycle control mechanisms.

CHAPTER II

REVIEW OF LITERATURE

Rhythmic sexual behavior patterns develop in female domestic animals during puberty. This behavior change is called estrus, and it occurs repeatedly unless pregnancy intervenes. The combination of physiological events which begin at one estrus period and end at the next is termed an estrous cycle (1).

Current knowledge of the estrous cycle is based on a small number of domestic and laboratory animal species. An accurate temporal account of the continuous physiological changes taking place in any one of these individual animals has not been possible. Temporal relationships therefore can only be inferred through data obtained from animals sacrificed at selected stages of the estrous cycle.

Three types of estrous cycles commonly observed, based on ovarian changes, have been outlined by Eckstein and Zuckerman (2). Their classification was based on the role of coitus in ovulation, and if the resulting corpus luteum was active or inactive. One type is exemplified in primates, cattle, sheep and swine (1, 3, 4, 5). The infertile cycle in these species culminates in spontaneous ovulation of mature follicles, and corpora lutea automatically form, become functional, and exist for a definite period of time. Species such as the rat provide an example of another type of estrous cycle where ovulation is spontaneous, but the corpora lutea which form are not functional unless mating has occurred (6, 7, 8). These estrous cycles are short (5 days) when rats

are not mated and longer (12 days) if cervical stimulation takes place. The third type of estrous cycle is one in which the maturation of pre-ovulatory vesicular follicles fails unless the male copulates with the female. The rabbit, cat and field mouse are examples of this type, and are commonly referred to as reflex ovulators (9, 10, 11). Smelser *et al*. (12) have shown that in the rabbit successive groups of follicles mature and degenerate rhythmically during the breeding season, and at any time there are a number of follicles capable of being ovulated when copulation occurs. The act of coitus in these species stimulates afferent neural pathways via the hypothalamus causing release of luteinizing hormone which in turn promotes the ovulatory process (13).

The estrous cycle in domestic animals may be divided into 4 phases as discussed by Asdell (14) and Emmens (5). These were called estrus, metestrus, diestrus and proestrus. Estrus was defined as the period of sexual receptivity, during which ovulation occurs in most species and the corpus luteum began its formation. Upon formation of the corpus luteum estrogen output is reduced and pituitary luteinizing hormone (LH) content declines (1,5). Metestrus was defined as the post-ovulatory phase in which the corpus luteum developed and began progesterone secretion, while extra-luteal estrogen secretion declined. Diestrus was defined as the period of sexual quiesence during which the influence of luteal progesterone on secondary sexual structures predominated. Proestrus marked the beginning of the estrous cycle, in which FSH dominantly influenced follicle growth, and culminated in rising estrogen levels and estrus. These criteria for classification of the estrous cycle are rather ill-defined for most domestic species. Proestrus is often referred to as the follicular phase, while estrus is denoted as the ovulatory period. Metestrus and diestrus together are referred to as the

luteal phase. It has been shown that the estrous cycle is in fact determined by a pituitary gland rhythm which is modulated by ovarian activity, external environment factors and probably uterine factors as well (3).

General Characteristics of the Porcine Estrous Cycle

A review of the literature revealed that considerable superficial data exists describing the estrous cycles of a number of species. In view of this, the present treatise will be confined to those studies related to the estrous cycle of swine with a few other species comparisons where pertinent.

Robertson et al. (15) and Burger (16) have reported respectively that the estrous cycle of swine averaged 20.5 and 21.7 days. Burger (16) found no correlation between estrous cycle length and duration of estrus in swine. McKenzie et al. (17,18) reported that age was not a factor in estrous cycle length.

The beginning of estrus in swine is a gradual phenomena. The detection of the precise time of the onset of estrus is difficult. It depends on many factors, chief of which is the change in behavior of male and female. Duration of estrus in female swine, based on acceptance of the male, has been shown to vary from 24 hours in gilts to 48 hours or more in mature sows (1,17). Breed and environmental temperature influence the duration of estrus. Burger (16) reported that duration of estrus was significantly shortened by high environmental temperatures, and that onset of estrus was not influenced by time of day or by coitus with intact or vasectomized boars.

Ovulation occurs in swine 24 to 36 hours after the onset of estrus and does not depend on coitus (16). Burger (16) has published a rather

comprehensive study on the time of ovulation in relation to estrus onset in swine. These observations showed that normal ovulation always occurred late in the estrus period regardless of its length. It varied with the breed. Further it was demonstrated, by using pentobarbital anesthesia and laparotomy techniques, that the length of time required for all Graafian follicles to rupture was 6.5 hours. Whereas Pitkjanen (19) reported that the average time period for completion of the ovulatory process was 2 hours. Hunter (20) recently demonstrated that in mature gilts, when treated with 500 IU of human chorionic gonadotropin (HCG) late in proestrus, the ovulation process lasted approximately one hour. Also he demonstrated that time of ovulation (40-42 hours) was predictable after HCG treatment. These reports indicated that the duration of the ovulatory process apparently varied considerably between individual animals.

Ovulation rate in the sow was reported by Lasley (21) to average 16.4 ova (range 10-25) per estrus. Robertson (15) reported that the number of ova shed varied significantly with each succeeding estrous cycle after puberty. Squires et al. (22), in comparing 273 post pubertal gilts with 72 mature sows, found that the average number of ova ovulated by gilts and sows were 11.5 ± 2.5 and 15.4 ± 3.4 respectively. These investigators calculated that in gilts each 10 day increase in age resulted in an increase of 0.35 ova.

Ovarian Changes During the Estrous Cycle

Few comprehensive accounts of the gross and microscopie ovarian morphological changes during the swine estrous cycle have been reported in the literature. The early works of Corner (23,24) have provided the most accurate and complete description of ovarian changes during the

cycle. Notations were made that 2-3 days before onset of estrus, Graafian follicles destined for ovulation began to enlarge rapidly and become very turgid. The theca interna hypertrophied and the ovum, with attached cumulus oophorus, partially separated from the granulosa layer of cells into the enlarged fluid-filled antrum of the Graafian follicle. Corner (24) observed that the ovum underwent the first stage of maturation just before ovulation in which the first polar body was released and the second polar spindle (metaphase) formed. After fertilization the second polar body was released. Spalding et al. (25) reported similar findings but related that the maturation process started about 5 hours prior to ovulation.

Robinson and Nalbandov (26) demonstrated that a highly significant positive correlation (0.69) existed between total anterior pituitary gonadotropin potency and ovarian follicular activity in swine. The average number of follicles rose suddenly on day 8 of the cycle. Total pituitary gonadotropin potency increased during the same period. However, these follicles were only 4-6 mm. in diameter and this size remained relatively static until day 18. Parlow et al. (27) observed a similar ovary-pituitary relationship in a group of 50 crossbred gilts. These investigators observed that follicles of ovulatory size (9-12 mm. dia.) appeared only at estrus (day 1). Nalbandov (28) stated that follicles of most mammals enlarge very little during the luteal phase and the early part of the follicular phase, but do undergo a surge in growth 1-3 days before expected ovulation.

Burger (16) described the mature Graafian follicle of swine as having a "seashell pink" appearance and that a transparent area appeared in the follicle membrane near the apex, and was associated with impending ovulation. In some cases the point of ovulation was evident in the

resulting corpora lutea up to day 12 of the estrous cycle. Burger (16) also noted that hemorrhagic areas in the vascular network of the follicle membrane, and the resultant extravasation of blood into the liquor folliculi, occurred frequently near the time of ovulation in swine. Nalbandov (28) reported that the mature follicle wall consists of 3 layers. As the outer layers separate during final preovulatory changes, the inner layer protrudes forming a papilla. Finally the outer layer also gives way and the ovum with attached cells flows out. On the other hand, Hunter (20) observed that proestrus follicles were turgid and about 7-10 mm. in diameter, while the follicle walls were clear colored and well vascularized. Just prior to ovulation (40 hours after HCG treatment) all follicles became flaccid indicating that the intrafollicular pressure had dropped. Hemorrhagic areas appeared in the follicle walls and the wall color progressed from a pink to a deep red color as ovulation neared. Hunter (20) showed that this change in color was primarily restricted to the follicle wall. No papillae were observed and ovulation itself was discrete. Complete structure collapse and recovery of ova were taken as positive evidence that ovulation had occurred. Espy (29) reported that a change in the collagen content of the follicular wall structure occurs near ovulation, and that ovulation itself may be dependent on enzymatic decomposition of this component resulting in the oozing process of ovulation. This investigator concluded that ovulation was not dependent on muscular or follicular pressure phenomena.

Corner (23) observed that with the collapse of the follicle at ovulation the granulosa cells, which remain intact, begin to hypertrophy and take on lipid material and become the primary lutein cells of mature corpora lutea. Corner (24) and Anderson (30) have shown that next

capillaries invade the granulosa lutein cells from the surrounding theca interna and carry some lipid containing cells from the theca interna with them. These theca lutein cells become dispersed among the granulosa lutein cells. Vascular growth and lutein cell differentiation are complete by day 7 of the cycle. Corner (24) observed that most corpora lutea are about 8-9 mm. in diameter by day 10, and that gross and microscopic evidence of regression of corpora appeared about day 15. At this time Corner (24) noted that granulosa lutein cells rapidly degenerated showing cytoplasmic vacuolation and pycnotic nuclei. Corpora lutea then decreased rapidly in size following degeneration of capillaries. Gradually the granulosa lutein cells were replaced by fibroblasts, while the theca lutein cells remained intact and became enmeshed in the forming connective tissue.

Burger (16) observed that, during diestrus in swine, ovarian weight increased after day 3 and reached a peak by day 12 of the estrous cycle. This was attributed to corpora lutea growth. Luteal tissue gradually changed in color from a dark red on day 3 to a pale purple by day 15. He observed that following day 15 corpora lutea rapidly changed from pale purple to a yellowish cream and then to a white color by day 18. A marked decrease in weight and vascularity was noted during this regressive phase. The size of corpora lutea decreased rapidly after day 18 and by day 40 most had regressed completely. On day 3 of the cycle the central cavities of freshly ovulated follicles were seen to contain blood clots (16). These clots were usually replaced by connective tissue plugs by day 6 or, in some cases, by a yellowish fluid which persisted until degeneration took place at day 18.

Anderson (30), following a very detailed study of cyclic variation in the lymph and blood vessels of sow ovaries, reported that the vascular

network of luteal tissue was very elaborate and that it developed in an organized fashion from the 2 layers of surrounding theca. This investigator also reported that lymphatic development occurred in a cyclic pattern, always following capillary development in luteal tissue and regressing before capillary involution at the time of luteal regression.

Bloor et al. (31) reported that the phospholipid content of the corpus luteum paralleled its development and regression. The phospholipid content rose rapidly from the 5th to the 10th day of the estrus cycle and declined rapidly after day 14. Boyd and Elden (32) and Barker (33) reported that the phospholipid content of porcine luteal tissue varied directly with the physiological function of the corpus as it developed and regressed, while the content of cholesterol varied inversely during the estrous cycle.

Similar observations on gross and histologic ovarian changes have been made in the cow and the ewe before, during and after ovulation (5, 14). Gross changes in corpora lutea were somewhat different due to the number and location of corpora lutea within the ovary as compared to swine. The corpora lutea of sheep reached maximum size by day 3 and at this time were reddish-pink in color. Corpora lutea became paler in color as diestrus progressed and after day 14 degeneration was very rapid (5). After ovulation in the bovine, granulosa cells hypertrophied and become filled with droplets of a yellow lipid material as the corpus luteum formed (5). Maximum size of the bovine corpus luteum was attained by day 16 and then it degenerated rapidly. The bovine corpus changed from a light brown color to a gold color by day 7, and then to a golden yellow by day 14. Between days 14 and 20 the corpus progressively changed from yellow to orange and then to a brick red. The brick-red color remained for months as the old corpus gradually involuted. These color changes have been associated with the quality and character

of lipid in lutein cells (34).

Uterine Changes During the Estrous Cycle

Histological studies of the uterine changes in domestic animals and women during different stages of the reproductive cycle were of primary concern during the second and third decades of this century.

A descriptive account of the gross uterine changes during the estrous cycle in swine have been described briefly by Corner (24). At estrus the opened uterus was paler than at other times of the cycle, and had a firm and slightly gelatinous inner surface (edema). If the uterus was opened on day 9 or 10 of the cycle the mucosa was consistently pink or red, and soft or velvety in appearance. Corner (24) also noted a periodic change in the external dimensions of the mid-cornual region of the uterus which were found directly related to the degree of edema. The uterus was always slightly larger just before and during estrus.

The most prominent morphological changes noted during the estrous cycle were observed in the endometrium and its associated glands. Corner (24) and McKenzie (18) observed that during estrus the surface epithelium of swine endometrium was pseudostratisfied and about 25-30 microns in height. The stroma was edematous and many neutrophils were present in the subepithelial layer. During the first 7 days after estrus the superficial epithelial cells hypertrophied and changed from pseudostratisfied to tall columnar-type cells. Three to four days after ovulation the endometrial gland cells also began to multiply, and by day 7 the basal cells became active. The sub-epithelial layer became infiltrated with greater numbers of neutrophils and eosinophils as the cycle progressed, while the edema lessened. High columnar cells predominated in the surface epithelium at days 8-10 and the surface became

characteristically wavy in appearance, and invasion of the superficial stroma with eosinophilic leucocytes became maximal. The surface epithelium reverted to a low columnar type epithelium between days 10 and 15 of the cycle. Cytoplasmic processes became evident during this period but were not thought to be cilia, since cilia normally exist only in the grandular cells and do not fluctuate with the cycle (24,18). From day 15 until the succeeding estrus, surface epithelial cells were low cuboidal and then became pseudostratified. Marked vacuolar degeneration of the epithelial cells was characteristic of this stage of the cycle. Thus the major changes noted during the estrous cycle appeared to be the cyclic manifestations of stroma edema, neutrophil and eosinophil infiltrations of the sub-epithelial areas, and changes in the growth of surface and glandular epithelium.

Similar observations on the cyclic changes in the endometrium of the ewe have also been reported by McKenzie _et al_. (35), Casida _et al_. (36) and Cole _et al_. (37). These investigators noted that edema and increased vascularity were evident in both cotyledonary and intercotyledonary areas at estrus and metestrus. Gland structure changes were prominent as mid-cycle approached, and folding of the epithelial surface was also observed.

Investigators generally agree that during the bovine estrous cycle the endometrial lining cells are tall and columnar during proestrus and estrus and then become low cuboidal by 2 days postestrus (38,39,40,41, 42). It has been further demonstrated that with development of the corpus luteum the height of these cells increased and reached a maximum by days 9-12, while the endometrial glands reached peak development at day 12. Retrogression of glandular epithelium appeared to begin about day 15 in the bovine (5).

In non-primates there appeared to be a cyclic sloughing of the endometrium much as described for primates, although the process does not involve loss of blood or any quantity of tissue since only the epithelial layer is involved (28). In the bovine and porcine, sloughing of the endometrial surface epithelium occurs late in the luteal phase (days 15-17) and has been shown to be nearly complete soon after day 18 (18, 38). This process of endometrial degeneration appears to be hormonally controlled by the ovary, although the control mechanism is not entirely understood (28).

The Anterior Pituitary Gonadotropic Hormones

It has been definitely established that a functional relationship exists between the anterior lobe of the pituitary gland and the ovary. Facts which support this relationship became evident once the techniques for hypophysectomy were perfected (43).

The existence of two separate gonadotropins was first postulated by Wiesner and Crew (44) following studies made by Smith and Engle (43). Evans and Simpson (45) reported that two substances were present in the anterior pituitary which specifically stimulated the ovary. However these workers believed that the substance with luteinizing activity was actually growth hormone. Fevold et al. (46) also separated two substances from dried sheep pituitary glands by using aqueous pyridine. One fraction had gonad stimulating ability and promoted follicular growth in the immature rat ovary, whereas the second substance had little effect on the immature rat ovary unless the follicle stimulating fraction was administered first. Since these early investigations, the terms follicle stimulating hormone (FSH) and luteinizing hormone (LH) have become commonplace in the literature.

Astwood *et al.* (47) reported that a third gonadotropin stimulated corpora lutea of the rat to produce progesterone. Later a substance was extracted from sheep anterior pituitary glands which also had a luteotropic effect on rat corpora lutea (48). Astwood (48) postulated that this factor was responsible for mammary growth in hypophysectomized rats, and for maintenance of luteal function in pseudopregnant rats. This substance, extracted from sheep anterior pituitary glands, was later identified as prolactin. Its luteotropic function has not been demonstrated specifically in the bovine, porcine, ovine or primate (49,50, 51).

The Anterior Pituitary-Hypothalamus Relationship

During the first three decades of this century reproductive physiologists were concerned with establishing the identity of the ovarian hormones, estrogen and progesterone, as well as the relationship of ovarian function to pituitary activity. Moore and Price (52) concluded that an anterior pituitary-gonadal relationship existed which explains gonad and accessory gland responses in mammals. As a result of their experiments, an end product "feedback" mechanism was postulated to exist between gonadal hormones and anterior pituitary gonadotropins. However this did not explain reflex ovulation, seasonal polyestrous animals, effects of light on estrous cycles, and the phenomena of pseudopregnancy in certain species.

The concept of neurohumoral control of the anterior pituitary through a secretomotor innervation was first perceived in the rabbit by Hinsey and Markee (53). Neural pathways from the hypothalamus were thought to first activate the posterior lobe of the pituitary, which in turn caused the anterior pituitary lobe to secrete the ovulating hormone.

Harris (54) found that local stimulation of the posterior hypothalamus and median eminence areas caused ovulation in the rabbit and ferret. It was also observed that lesions of the pituitary stalk promoted genital atrophy in both male and female rabbits. Brooks (55) confirmed the findings of Harris on ovulation, but suggested that diminished sexual activity or genital atrophy did not necessarily follow these leisons.

The anatomy of the hypophyseal-portal system was first described by Popa and Fielding (56,57) and further substantiated by Green (58). Green (58) studied 76 species including man. These very detailed studies gave strong support to the theory of secretomotor innervation. Green and Harris (59) described the vascular connections between the hypothalamus and anterior pituitary in various species using india ink infusions. These workers were able to definitely establish the anatomy of the nervous and vascular connections between the hypothalamus and adenohypophysis as well as the direction of blood flow. It was postulated that humoral substances (releasing factors) were released from special nerve endings which terminated in close proximity to the primary capillary plexus of the median eminence. These substances were then transported by the hypothalamo-hypophyseal portal vessels to the anterior pituitary lobe where release and/or increased synthesis of certain gonadotropic hormones took place. This postulation gained further support when it was demonstrated that section of the pituitary stalk prevented the anterior pituitary from performing its normal function (60). Pituitary transplantation studies by Harris and Jacobsohn (61) and Nikitovitch-Winer (62) have also substantiated the dependence of the pituitary on the hypophyseal portal blood supply for its function. Their experiments revealed that transplanted anterior pituitary glands lacked secretory activity except for prolactin.

The early concept of central nervous system involvement in anterior pituitary gland function stemmed from observations that environmental factors such as light, ability of copulation to promote ovulation and suckling of young were able to alter the estrous cycle (63,64). It has also been shown that the influence of gonadal steroids on gonadotropin secretion can be either inhibitory or stimulatory depending on concentration and stage of the estrous cycle. Recently a concept was developed which states that neural control of gonadotropin release is altered not only by environmental influences, but by the continuous influence of temporal changes in the menstrual or estrous cycles as well. Thus the view has developed that the hypothalamus controls the pituitary and that certain centers in the hypothalamus are involved in luteinizing and follicle stimulating hormone release (65). This theory of hypothalamic control is based primarily on experimental evidence in rats.

McCann and Ramirez (66) observed that if lesions were made in certain rostral areas just dorsal to the optic chiasma in the hypothalamus of rats, certain vaginal changes occurred typical of estrus. Follicular growth in these rats was prominent, but ovulation did not occur. Apparently estrogen was secreted since a persistent estrus type vaginal smear and an enlarged uterus were evident. The pars distalis of these rats was also enlarged. Thus the postulation was made that FSH and LH were being secreted at a constant rate. There appeared to be a partial defect in synthesis and release of LH such that the ovulatory burst of LH release did not occur. On the other hand, if lesions were placed in the median eminence area of the hypothalamus the estrous cycle disappeared and the animals remained in constant diestrus. Large functional corpora lutea persisted, and no follicles developed. Apparently LH and FSH secretions

curtailed by these lesions. Further, ovariectomy failed to elevate levels of plasma LH which were normally seen in intact rats (66).

The persistent functional corpora lutea observed in the constant diestrus syndrome noted above was shown to be due to prolactin (48). This supported the postulation that prolactin was luteotropic in the rat. Thus when hypothalamic lesions were placed in the median eminence area, the connections between the hypothalamic centers and the anterior pituitary gland were broken and secretion of FSH and LH were curtailed, while LTH (prolactin; luteutropic hormone) secretion was enhanced due to the lack of prolactin inhibitory factor (66,67).

If testicular tissue was transplanted into the newborn female rat it was found that these rats, on maturing, became anovulatory and showed persistent vaginal cornification (68). Work by Barraclough (69) in which steroid hormones (1250 ug testosterone propionate) were injected as late as 5 days after birth produced similar results. Pituitary glands taken from male rats or mice and transplanted beneath the median eminence of hypophysectomized female rats or mice, restored normal estrous cycles (70). In another experiment electrical stimulation of the hypothalamus of androgenized female rats caused LH secretion by the anterior pituitary (69). This suggested to these investigators that sex differences in gonadotropin secretion was not within the anterior pituitary but at the hypothalamic level. Numerous investigators have reported that the general area of the hypothalamus just dorsal and anterior to the median eminence appears to be essential for mediating gonadotropic release. Electrical lesions or estrogen implants in the arcuate nucleus region (dorsal to median eminence) have produced gonadal atrophy in the rat (71,72). However, stimulation of this arcuate nucleus area, both in the normal and in the androgenized female, produced

ovulation (73,74,75).

Gorski (65) and Barraclough (75) proposed a theory of dual hypothalamic control of adenophypophyseal gonadotropin secretion based on numerous experiments. These workers proposed that the arcuate-ventromedial nucleus just dorsal to the pituitary median eminence region regulated the first level of hypothalamic control or "tonic" discharge of gonadotropins (FSH and LH). This discharge of gonadotropins, though sufficient to maintain estrogen secretion in the female, is not sufficient to initiate ovulatory surges of gonadotropin (LH). A higher or "cyclic" center located in the anterior hypothalamus just dorsal to the optic chiasma regulates the second level of control in the female ovulatory process. Further it was postulated that the "cyclic" center exerts an integrating function on the "tonic" center during the estrous cycle such that certain critical gonadal steroid concentrations, neurogenic stimuli, and other external environmental stimuli (light) are integrated. During the norman estrous cycle in female rats, the preoptic area responds on the day of proestrus when certain environmental (light) and hormonal circumstances (estrogen-progesterone) synergize to facilitate stimulation of the arcuate-ventromedial nuclei causing release of neurohumors (especially LH-releasing factor). An ovulatory discharge of LH from the adenohypophysis follows then and a new estrous cycle is initiated (75).

Phoenix et al. (76) reported that steroid hormones in the adult guinea pig act as activators or inhibitors of existing hypothalamic neural patterns. When androgens were given prenatally to guinea pigs the sexual behavior pattern of the female was organized into the acyclic pattern of the male.

Barraclough et al. (77) have suggested that certain low doses of

progesterone may act on the anterior hypothalamic preoptic "cyclic" center to facilitate ovulation. Whereas in androgenized rats, or in rats with large preoptic lesions (cyclic center), the threshold became too great to permit appropriate transmission of the triggering stimulus to the infundibular nuclei (tonic center). Therefore progesterone became ineffective.

Barraclough (75) concluded that normal differentiation of the hypothalamic regulatory processes occurred early in the postnatal life of the female rat and that gonadal hormones were not involved. As a result, the neural elements which regulated anterior pituitary gonadotropin synthesis, storage and release, functioned in a cyclic pattern. He further suggested that intrinsic stimuli were transmitted from the "cyclic" center via neural pathways, which have a lowered threshold due to critical ovarian steroid blood levels and other neural stimuli, to the "tonic" hypothalamic center. A decrease in threshold excitability occurred once during the estrous cycle although the potential stimulus appeared once every 24 hours in the rat. The proposition was made that progesterone acted synergistically with estrogen to lower the threshold of excitability. Evidence that ovarian progesterone increases during proestrus in the rat has been shown by Telegdy and Endroczi (78). Thus certain steroid ratios may be a key factor in the ovulatory process.

The Anterior Pituitary-Gonad Relationship

The primary target organs of the pituitary gonadotropins are the ovary and testis. In the male, FSH, LH and the androgens are all involved in the normal development and function of the testis. Luteinizing hormone acts to stimulate the interstitial cells to produce androgens, while FSH is concerned with tubule physiology (79). The growth

and development of ovarian follicles in mammals is dependent upon FSH, but LH is essential for follicle maturation. Both FSH and LH are essential for the synthesis of estrogen. Rising estrogen blood levels suppress release of FSH and facilitate release of LH. Purified LH in itself has no conspicuous effects on the ovarian follicle (79). Thus it is well established that FSH promotes ovarian growth and follicular maturation, while LH is essential for estrogen synthesis, ovulation and initial development of the corpus luteum in some species (79).

Nalbandov (28) indicated that FSH and LH were secreted continuously by the anterior pituitary throughout the estrous cycle, but the proportions of each change during the different stages of the cycle. Greep *et al.* (80) noted, in experiments dealing with the effect of purified porcine FSH and LH pituitary fractions on ovarian and uterine weights of immature hypophysectomized rats, that purified FSH fractions alone caused increased ovarian weight, but did not initiate an increase in uterine weight. This suggested to these workers that FSH was not capable of stimulating estrogen production in the ovaries of swine. However, in normal preovulatory intact animals uterine and vaginal changes were observed. These observations supported the contention that the anterior pituitary continuously secretes both gonadotropins.

Estrogen has been found to inhibit FSH secretion by the anterior pituitary. Large quantities of estrogen completely inhibit the secretion of gonadotropic hormones (28). In female rats, large amounts of estrogen can lead to complete arrest of the physiological and morphological development of the ovary. Hypophysectomy will cause similar changes. On the other hand, it has been shown experimentally that injection of low levels of certain estrogenic substances can cause ovulation in cattle, sheep, rabbits and rats (28). It has also been reported

that progesterone can cause the release of LH (81). Thus it seems apparent that both steroids, when present in a critical ratio at a certain time in the hypothalamus may be essential in the ovulatory process in mammals (75).

The formation, maintenance and function of corpora lutea as described for the rat and mouse cannot be related entirely to other mammals, since prolactin has been shown to be non-luteotropic in the rabbit (82), cow (83), woman (84) and sow (85). Sammuelwitz (86) demonstrated that injections of high doses of progesterone caused severe degeneration of corpora lutea in pregnant gilts. However, if high levels of progesterone were given at or near ovulation and continued throughout the luteal phase of non-pregnant gilts, corpora lutea functioned normally. Brinkley (87) concluded that the porcine corpus luteum needs no sustained pituitary gland stimulation beyond that received at ovulation for normal growth and function throughout the estrous cycle. On the other hand, Kilpatrick _et al_. (88) have presented evidence which indicates that LH may be luteotropic in the rabbit, while Hansel (89) postulated from his experiments that LH may be luteotropic in the bovine. Precise evidence has not been established for the existence of a luteotropic hormone (LTH) in any of these species.

The Currently Accepted LH and FSH Bioassays

Early investigators evaluated pituitary-ovarian relationships during the swine estrous cycle by using total gonadotropin bioassay techniques. Wolfe (90) showed that swine anterior pituitary tissue from various stages of the estrous cycle induced certain measurable changes in mature female rabbit ovaries. It was found that 1 mg. of fresh pituitary tissue taken during the proestrus phase was needed to induce

ovulation in mature rabbits. However it took 40 mg. of tissue from diestrus periods and 10 mg. from estrus periods to induce ovulation in mature female rabbits. Variable amounts of pituitary tissue taken from pregnant swine were required to induce ovulation in rabbits.

Greep *et al.* (80) demonstrated that the weight response of the anterior lobe of the prostate gland of the male rat bore a quantitative relationship to the dose of LH given and this response was not potentiated by FSH. This observation became the basis for a bioassay procedure (ventral prostate assay) which has been used extensively by many investigators for measuring LH in pituitary tissue. Recently Parlow (91) developed the ovarian ascorbic acid depletion assay which has been shown to be more sensitive for measuring LH activity (80).

Robinson *et al.* (26) assayed the total gonadotropin potency of 33 sows by collecting anterior pituitary tissue at various periods of a 21 day estrous cycle. The relative potencies were determined from the testicular weight response in day-old chicks. It was reported that the gonadotropin hormone content was lowest during estrus and remained low until day 8 of the estrous cycle. After day 8 the potency increased suddenly and then remained at a higher level until day 20. The prime observation noted when correlating total gonadotropin content with ovarian morphological changes was that a high positive correlation existed between day of cycle and potency ($r = 0.62$), and between potency and number or size of follicles in ovaries ($r = 0.69$). These investigators could not demonstrate an association between pituitary weight and rate of hormone secretion. Thus it was concluded that the bioassay of anterior pituitary glands in swine reflected the rate at which the gonadotrophic hormones were being secreted.

Hollandbeck *et al.* (92) evaluated the pituitary gland potency of 46

female swine varying in age from 1 to 1330 days using the day-old chick testes weight as the assay end point. Prepuberal porcine anterior pituitary glands manifested high potencies and this potency declined as puberty neared. After puberty the gonadotropin potency stabilized and then varied only in a cyclic manner. It was proposed that the high gonadotropin potency observed in the pituitary glands of young animals was caused by the high rate of FSH secretion, and that as the FSH/LH ratio reached a certain lower optimum level puberty resulted.

Day _et al_. (93,94) reported on the bioassay of anterior pituitary glands from 30 gilts slaughtered during estrus, diestrus, early proestrus and during the 25th and 85th day of pregnancy for FSH, LH and lactogenic hormone. Follicle stimulating hormone was assayed using the uterine weight response of hypophysectomized immature rats. The ventral prostate assay was used to evaluate LH potency, while the pigeon crop gland assay was used to evaluate lactogenic hormone potency (80,95). Potency of FSH increased significantly on days 10-12 after estrus and during the proestrus period. Potency was higher on day 85 than on day 25 of pregnancy. Relative LH potency was higher in pregnant than in non-pregnant animals. Lactogenic hormone potency was lowest near ovulation and highest at the 85th day of pregnancy in swine.

Parlow (96), using three bioassay methods, assayed pituitary gonadotropins in the rat, and showed that the simultaneous use of bioassays for FSH and LH provides more specific information on estrous cycle variations than nonspecific bioassays or a single specific bioassay.

Parlow (91) has developed a highly sensitive, specific and relatively precise bioassay technique which can be used to measure LH activity in tissue or plasma. Immature female rats are first pretreated with high doses of pregnant mare serum followed in 60 hours with large

doses of human chorionic gonadotropin. The ovaries of these immature rats become large and heavily luteinized. Preparations of LH may be assayed quantitatively by intravenous injection 6 to 8 days following treatment of these rats with HCG. Four hours later the ascorbic acid content of the luteinized ovaries is measured. The ovarian ascorbic acid depletion response is proportional to the log of the LH dose.

Simpson et al. (97) showed that human chorionic gonadotropin (HCG) augments the action of FSH on the ovary to increase ovarian weight. Steelman and Pohley (98) developed a currently accepted bioassay for FSH from these observations. Immature female rats are injected subcutaneously with test solutions to which a standard amount of HCG has been added. These rats are sacrificed on the fourth day after a three day schedule of injections, and the ovarian weight is determined. Human chorionic gonadotropin appears to augment the FSH response and overrides the response to any LH which may contaminate the samples. This assay has been shown to be relatively precise and specific for FSH.

Igarashi and McCann (99) have developed a mouse uterine weight assay method based on the HCG augmentation principle which has been reported to be highly sensitive to, but not specific for FSH. When LH levels are low, as in blood plasma, this method appears to be specific enough to measure FSH activity reasonably well. This bioassay method has been used to measure plasma concentrations of FSH in some species (100).

Anterior Pituitary Tissue FSH - LH Concentrations

Parlow et al. (27), using the HCG-augmentation assay for FSH and the ovarian ascorbic acid depletion assay for LH, observed that FSH and LH concentrations were low in porcine anterior pituitary glands between

days 1 and 4 of the estrous cycle and thereafter increased sharply between days 4 and 10. This trend corresponded with the increase in the number of follicles which were 4 to 8 mm. in diameter. Increased release of pituitary FSH and LH into the blood, estimated on the basis of follicular growth, was correlated with pituitary FSH and LH concentrations under two situations. First, it was correlated with increased pituitary FSH and LH content between days 4 and 10, and second, with a marked decrease in pituitary content between days 18 and 1 of the estrous cycle. Mean FSH concentrations (ug./mg. dry pituitary) varied from less than 7 ug. on day 1 to 19.3 ug. on day 16. Luteinizing hormone (LH) varied from a low of 0.7 ug. on day 1 to a high of 2.2 ug. on day 18 of the estrous cycle. Follicle stimulating and luteinizing hormone concentrations in pituitary glands of 25-day pregnant gilts were significantly higher than those of the estrous cycle, being 24 ug. and 4.9 ug. per gram of dry pituitary, respectively.

Melampy (101) found that FSH concentrations in swine pituitaries did not vary significantly throughout pregnancy and lactation. Luteinizing hormone concentrations increased from 13 (3.6 ug./mg. dry pituitary) to a maximum at day 18 (4.7 ug./mg.) of pregnancy and then gradually declined throughout gestation. The anterior pituitary of the pregnant sow was found to have twice the gonadotropin potency reported by Parlow (27) during the estrous cycle. This suggested to Melampy (101) that a change in pituitary function was associated with pregnancy. It was not determined whether the increase was due to a greater rate of synthesis or reduced release.

Anderson _et al._ (102) analyzed the pituitary FSH and LH activities of pigs in which corpora lutea were enucleated at day 6 of the estrous cycle. These animals were sacrificed on various days following luteal

enucleation and/or progesterone treatment. Anterior pituitary FSH concentrations dropped 24 hours after enucleation, but rose again when estrus appeared 6 days later. Luteinizing hormone remained constant after enucleation but dropped at estrus 6 days later. When progesterone was given after enucleation, the following estrus was delayed. In the intact animals, progesterone caused both FSH and LH to increase in the pituitary gland, but after the treatment ended estrus occurred in 6 days and pituitary FSH activity decreased while LH did not. If pigs were hysterectomized on days 7 or 8 of the estrous cycle and corpora lutea enucleated 25 days later, pituitary LH was markedly decreased at the onset of estrus 6 days following enucleation (102). It was postulated, since numerous follicles 4-6 mm. diameter appeared in those pigs with enucleated corpora lutea but progesterone treated and in hysterectomized pigs, that pituitary FSH was released into the circulation during the estrus inhibition by progesterone. Rothchild (81) demonstrated in the rat that a positive relationship existed between circulating levels of progesterone and prolactin, while a negative relationship existed between progesterone and LH. After withdrawal of progesterone treatment, pituitary LH decreased in 4 or 5 days to levels which are similar to levels usually found in proestrus. A similar decline was noted by Anderson (102) for those pigs in which corpora lutea were enucleated only as well as in those that were hysterectomized. However, no significant decrease was noted after withdrawal of progesterone treatment.

Robertson and Hutchinson (103) determined the levels of FSH (Steelman-Pohley assay) and LH (Parlow assay) in the pituitary of ewes sacrificed at known stages of the estrous cycle, of pregnancy and of lactation. These changes were correlated with the morphological status of the ovary. The size and number of follicles (4 mm. diameter and

over) increased early in the luteal phase (day 5). The pituitary FSH potencies dropped significantly between the 4th and 36th hour after the onset of estrus. It was observed that the pituitary content decreased by an amount approximately equal to that found during the luteal phase of the estrous cycle. The pituitary content of FSH reached a steady level by day 5 and remained constant until day 15 after which a steady rise occurred until the 4th hour following the onset of estrus. Pituitary LH potency dropped significantly within 36 hours after the onset of estrus. This drop was followed by a progressive rise in pituitary LH potency to the preovulatory level. There appeared to be relatively less LH than FSH in the anterior pituitary gland during anestrus in ewes. Robertson and Rakha (104) reported a difference in the timing of the release of FSH and LH from the anterior pituitary of sheep at ovulation. These investigators observed that pituitary FSH began to decline 8 hours before the onset of estrus and declined 52% within 6 hours after estrus, whereas the pituitary LH content began to decrease at the beginning of estrus and reached its lowest level by 6 hours after estrus. The concentration of LH in the anterior pituitary also became depleted by 52% at this time. The concentration of FSH varied from 4.83 ug./mg. of dry pituitary (NIH-FSH-S1 equivalent) 12 hours before estrus to 1.93 ug./mg. of pituitary 6 hours after estrus. On the other hand, 17.2 ug. of LH/mg. dry pituitary (NIH-LH-S1 equivalent) was measured 12 hours before estrus and this decreased to 6.6 ug./mg. of pituitary 6 hours after estrus. These workers concluded that these rapid changes in pituitary FSH and LH potencies indicated that the release of gonadotropic hormones occurred during a precise period of a few hours. Their studies also suggested that as in the cow, LH-FSH ratios varied with the stage of the estrous cycle and that this variation was due to a differential time sequence of

release (105).

A significant drop in pituitary FSH and LH concentrations has been shown by Rakha and Robertson (105) to occur in the cow during the period 0-18 hours following the onset of estrus. Bovine pituitary LH potency rose to a peak of 21.2 ug./mg. dry pituitary (NIH-LH-B1 equivalent) just prior to the onset of estrus, while FSH pituitary content reached a peak of 1.83 ug./mg. dry pituitary (NIH-FSH-S1 equivalent). A 27% decrease in anterior pituitary content followed at the termination of estrus as well as a 61% decline in LH potency. They postulated that FSH as well as LH played a role in the induction of ovulation in the cow. Calculation of LH-FSH ratios in cows indicated to these investigators that LH-FSH ratios varied considerably during the estrus period.

Everett _et al._ (106) demonstrated that in rats subjected to a controlled lighting schedule (lights from 5 AM to 7 PM) that a preovulatory surge of LH was released between 2 and 4 PM on the afternoon of proestrus. Ovulation then occurred at 1 to 2:30 AM the following night. Mills (107) showed that a drop in pituitary LH concentration of the rat between 10 AM and 4 PM on the day of proestrus was associated with LH release. Schwartz (108), reporting on the changes in pituitary LH content during the rat estrous cycle, found the most significant drop in pituitary LH (6.3 ug. to 3.6 ug./pituitary) took place between the mornings of proestrus and estrus. Schwartz (108) proposed that in order for LH release and ovulation to occur, the existent daily facilitation of the "cyclic" (anterior hypothalamic preoptic nucleus) center must be supplemented by another facilitatory factor which occurred only on the day of proestrus. It was proposed that this additional factor may be a specific ratio of estrogen-progesterone feeding back on these hypothalamic centers.

Greenwald (109), in studies correlating ovarian follicular development with pituitary FSH and LH content of the pregnant rat, observed that pituitary LH potency increased three-fold between days 1 and 8, but thereafter remained relatively constant until delivery of the young. However, the FSH-LH ratio increased significantly after day 12 of pregnancy and reached a peak just before delivery.

Schwartz and Rothchild (110) have reported from their experiments that pituitary LH concentrations averaged 5.4 ug./mg. of pituitary tissue on days 9 through 11 of pseudopregnancy. Recently Greenwald (109) reported that this decline did not occur in pregnant rats until at least day 21 (parturition). van Rees and De Groot (111) showed that the pituitary FSH content was significantly increased by day 7 of pseudopregnancy, but not in control estrus rats. However, blood levels of FSH were the same in both groups. Pituitary FSH potency increased from 29.6 ug./mg. (NIH-LH-S1 equivalent) pituitary on day 12 of pregnancy to 62.6 ug. on day 22.

Greenwald (109) also reported that a significant drop in pituitary FSH occurred after parturition in rats and that rising progesterone blood levels were responsible for the slight increase in pituitary LH content during pregnancy. Further, he postulated that progesterone may enhance FSH synthesis by its direct action on the pituitary, or that estrogen blocked the release without altering synthesis. From indirect evidence a postulation was made that circulating levels of estrogen increased during the last 7 days of gestation. However estrogen blood levels were not precisely measured.

In a later experiment, Greenwald _et al_. (112) observed that pituitary FSH and LH concentrations in pregnant and lactating hamsters were different than those reported for the rat or pig (109,101). Pituitary

FSH was found to decrease steadily during the first half of pregnancy and to reach its lowest value at day 8. A constant pituitary level existed until day 14 of lactation. Peak values of LH were reached on day 12 of pregnancy and then the pituitary content declined, which coincided with elevated plasma levels. Follicle stimulating hormone potency decreased and LH potency increased sharply after suckling ceased.

Prolactin and FSH were found to be luteotropic in the hamster, whereas LH modified this relationship to the extent that high doses were anti-luteotropic if the pituitary FSH potency was low (113). It was also noted that following parturition in the hamster, pituitary FSH increased and LH decreased, while plasma LH increased. Prolactin was observed to increase soon after parturition. Corpora lutea regressed, while prolactin and LH predominated during lactation.

Rothchild (81) reported that, in the rat, sheep, pig and cow, the interval from the period of lowest pituitary FSH and LH potency (around estrus) to the period of maximum pituitary potency was approximately 7-10 days. The proposal was made that the increase in pituitary potency was induced by the rising blood progesterone levels. This suggested that either an increase in the rate of gonadotropin formation and/or a decrease in gonadotropin release was initiated by the progesterone.

Blood Plasma FSH - LH Concentrations

Ramirez and McCann (114) successively measured blood plasma levels of LH during the rat estrous cycle. They found that blood plasma LH levels varied from 1.9 ug./100 ml. of plasma during diestrus to 11.4 ug. on the P.M. of proestrus. These results showed that plasma LH was highest when estrogen secretion was also at its peak as noted by the elevated uterine and ovarian weights. These values supported the concept

of a proestrus surge of LH in the rat.

Blood levels of gonadotropic hormones have been measured in women (115), mares (116) and ewes (117). None of these investigators used the Parlow OAAD assay (91) to measure plasma LH potency, and none quantitated plasma FSH. Anderson and McShan (118) outlined a method of fractionating blood plasma in which LH may be concentrated and thus be measured by the OAAD assay. These workers obtained blood plasma samples at selected periods during the estrous cycles of sows, cows and rats. Their results suggested, although a very limited number of animals were involved, that the concentration of LH in blood plasma rises rapidly 12 hours before ovulation in gilts (10.95 ug./100 ml. plasma), 6-17 hours before ovulation in the cow (11.4 ug./100 ml. plasma) and 6-12 hours before ovulation in the rat (16.83 ug./100 ml. plasma), while plasma LH was low at other times during the estrous cycle.

Liptrap and Raeside (119), using the ovarian cholesterol depletion assay method for measuring LH activity in the blood of swine, observed that a marked elevation of blood plasma LH activity occurred along with a peak in urinary estrogen excretion about 40-48 hours prior to ovulation. This was in contrast to the values reported by Anderson and McShan (118) but in agreement with the results reported for the rat (114). Liptrap and Raeside (119) also suggested that the close correlation which existed between peak LH activity in blood plasma and urinary estrogen levels gave support to McCann's (120) postulation that estrogen or a certain estrogen-progesterone ratio caused the ovulatory surge of LH.

Igarashi _et al._ (100) recently reported on the measurement of serum FSH levels during the normal menstrual cycle in women. They attempted to measure the change in human blood FSH levels during the normal

menstrual cycle using the Igarashi-McCann (99) FSH bioassay technique. FSH activity was assayed in 1.5 ml. of unextracted serum. FSH levels were high during the follicular phase and not detectable in the late luteal phase. Serum FSH peaked on days 8 and 11 (426.3 ug. of NIH-FSH-S2 equivalent per 100 ml. serum) of the menstrual cycle. Igarashi et al. (100) also reported that serum LH levels in women varied from 31 to 98 ug./100 ml. serum (NIH-LH-S1 equivalent) during the follicular phase, 87 to 216 ug./100 ml. serum in the ovulatory phase and 72 to 29 ug./100 ml. serum in the luteal phase. Serum FSH and LH potencies clearly showed a greater lack of correlation than implied by the pituitary gonadotropin ratios in other mammals.

Progesterone and Luteinizing Hormone Relationships

Neill et al. (121) studied the temporal relationships between plasma levels of LH and progesterone during the normal menstrual cycle of women. Plasma samples were assayed daily for progesterone concentrations (chemical assay) and for LH concentrations (radioimmunologic assay). Progesterone concentrations were consistently low (.2-1.8 ng./ml.) during the first half of the menstrual cycle and did not begin to rise until after the preovulatory surge of plasma LH was evident. After the decline in plasma LH, plasma progesterone increased rapidly until a maximum of 10-19 ng./ml. was reached in 3 to 5 days following the preovulatory LH surge. Progesterone remained at this elevated level for another 4-6 days. Progesterone levels decreased rapidly 24 hours before menstruation without comparable changes in plasma LH. They suggested that preovulatory progesterone was dependent upon LH activity much as Rothchild (81) has proposed. Further, a secondary rise in plasma LH concentration was observed on the descending limb of the LH peak just

prior to ovulation. It was proposed that this secondary rise in plasma LH was due to the rapidly rising progesterone levels. The existance of a negative-feedback mechanism, between rising plasma progesterone levels and LH levels, seemed uncertain at this time.

An interesting study was made by Corbin (122) on the effect of median eminence implants of LH in ovariectomized rats on pituitary and plasma LH levels. Consistently rats with LH implants in the median eminence showed a significantly lower pituitary LH content. Plasma levels in these rats were not significantly different. It was proposed that LH played a role in the negative feedback control of its hypothalamic regulator (LH releasing factor).

It has become apparent from the many investigations cited here that progesterone may be the key effector in the secretory control of the pituitary gonadotropins. What critical relationship exists between progesterone and/or other factors on the hypothalamic centers has not been determined. Most certainly progesterone in sufficient doses can exert its effect on the estrous cycle by inhibiting ovulation. Progesterone does this without altering basal follicle growth and estrogen secretion (81). The ovulation inhibiting effect has also been shown to be associated in certain mammals with either no effect, a definite increase or a definite decrease on pituitary FSH-LH potency. Rothchild (81) believed that progesterone did not interfere with the tonic secretion of FSH or LH. However the precise quantities of these tonic levels were not known.

Growth, Maintenance and Regression of Corpora Lutea

Rothchild (81) reported that corpora lutea of most polyestrous mammals exhibit a common pattern of growth, progesterone secretion and

regression during the estrous cycle. Corpora lutea matured usually by 7 to 10 days after ovulation and were then maintained for another 4 to 7 days before regressing. For most domestic species luteal regression begins not later than 2 weeks after ovulation. An increase in size and number of granulosa cells was the main form of growth seen in the developing corpora lutea, although the proportion of granulosa lutein and theca lutein cells appeared to vary somewhat among species. For example, in women the theca cells were more prominent in the mature corpus luteum than in swine corpora lutea. Luteal tissue progesterone concentration has been shown to closely parallel luteal growth as well as blood plasma levels in swine (123,124). Generally luteal progesterone concentrations decreased, while other biochemical and cytochemical signs of regression appeared before gross signs of regression were seen (24,32,125). Luteal regression appeared to be an irreversible process once initiated. Luteal physiology, therefore, consists of one to two weeks of growth and secretory activity followed by regression and repetition of the cycle. Clearly two processes were involved in the luteal cycle. The first process was that which initiated and maintained growth and secretory function and which has been defined by Rothchild (126) as the luteotrophic process. The second process was concerned with "shutting off" progesterone biosynthesis, and the degeneration of luteal tissue, defined by Short (127) as the luteolytic process.

It was shown originally by Dresel (128) in the mouse, and by Astwood *et al.* (48) in the rat, that corpora lutea do not grow or secrete progesterone in the absence of the anterior pituitary gland. These investigators also found that during the normal estrous cycle in these species, luteal growth and progesterone biosynthesis were low. If certain stimuli such as coitus or mechanical stimulation of the cervix were

applied, then luteal growth and progesterone synthesis increased and the cycle lasted about 13 days. This activity was identified with prolactin.

Prolactin has been found in the anterior pituitary gland of most mammals, and it was one of the first anterior pituitary hormones to be isolated in a purified form (129). Dresel (128) and Astwood (48) reported that luteotropic activity was not present when other gonadotropic hormones were used in the rat. These observations have been substantiated by other investigators (130). Rothchild (126) discussed the status of prolactin in relation to its luteotropic effects in other mammals. He stated that the duration of luteal tissue function is not increased beyond the limits of the luteal phase in women, rabbits, ewes, cows or gilts after prolactin treatment. It has also been demonstrated that prolactin does not prevent the immediate or eventual regression of corpora lutea after hypohysectomy (88). Corpora lutea of gilts, cows, women and guinea pigs were found to grow and secrete progesterone for a certain period of time even in the absence of the anterior pituitary gland (131,132,133,134). Other investigators have shown that when prolactin was added to luteal tissue in vitro, progesterone synthesis was not increased significantly (85).

It has been reported that sectioning the pituitary stalk in most vertebrates causes continuous prolactin secretion (81). When the pituitary stalk was sectioned in the pregnant goat, corpora lutea regressed and abortion followed, while exogenous progesterone would maintain pregnancy (135). This suggests that prolactin was not luteotropic.

Rothchild (126) postulated that failure to demonstrate luteotropic properties of prolactin in species other than the rat and mouse may be due to: (a) need for synergism with other gonadotropic hormones; (b)

presence of unknown factors which alter the responsiveness of the corpus luteum; (c) species specificity of prolactin; and (d) differences of *in vivo* and *in vitro* systems. This investigator postulated that the luteotropic process was not the result of a single hormone action, but rather the result of a combination of events occurring during the estrous cycle in which the interaction of several hormones (prolactin included) bring about the luteotropic process.

Evidence presented in the literature indicates that other hormones do have luteotropic properties under conditions in which prolactin does not. Estrogen or LH will maintain corpora lutea of hypophysectomized rabbits (88,136). Luteinizing hormone or FSH has been found to stimulate progesterone secretion by ovine corpora lutea *in vivo*, while LH will stimulate bovine luteal tissue *in vitro* (137). However, Denamur and Mauleon (138) were unable to prolong corpora lutea life in hypophysectomized sheep with LH or with LH-ovine prolactin combinations. Short (127) concluded that neither LH nor prolactin were luteotropic in sheep. On the other hand, LH appeared to prolong the functional activity of corpora lutea of hypophysectomized-hysterectomized sows (139).

Donovan (140) demonstrated that sectioning the pituitary stalk in the ferret after ovulation resulted in normal luteal function, while prolactin treatment delayed regression in hypophysectomized ferrets. Denamur and Mauleon (138) showed that sectioning of the pituitary stalk in sheep does not shorten luteal function (16 days), but if hypophysectomy was performed instead, then luteal function lasted only 10 days. If the pituitary stalk was sectioned in hysterectomized ewes, then corpora lutea functioned for at least another 20 days, whereas hypophysectomy terminated this function within 5-7 days. Thibault (141) has shown that in hypophysectomized-hysterectomized sheep, prolactin prolonged

luteal activity. Rothchild (126) suggested that the failure of prolactin to prevent regression of corpora lutea in hypophysectomized sheep may be due to the overriding luteolytic effect of the uterus.

Eckstein and Zuckerman (142) suggested that the 6 week period of pseudopregnancy commonly observed in the bitch following estrus was associated with prolactin secretion since the mammary glands show secretory activity. Similarly, in the post-parturient cat, the corpora lutea of pregnancy persist and show signs of being functional. These corpora lutea regress rapidly when suckling is prevented.

Estrogen has been shown to have luteotropic properties in swine (143). However in swine, as in rats and mice, the anterior pituitary appears to be essential.

Rothchild (144) postulated that progesterone itself may be involved in the luteotropic process. The suggestion was made that progesterone augmented the action of prolactin in the hypophysectomized-autotransplanted pituitary rat and that progesterone augmented growth of corpora lutea.

Moor and Rowson (145) showed that the most obvious luteotropic stimulus of all was the presence of an embryo in the uterus. A 12 day embryo when transferred to the uterus of a nonpregnant recipient ewe on day 12 of the estrous cycle resulted in a normal pregnancy. However if this transfer was made on day 13 the corpus luteum regressed as usual and the next estrus period followed. If embryos were flushed out of the uterus between days 5 and 12 of the cycle the normal events of the estrous cycle followed, but if flushing was performed on days 13 and 14 then the estrous cycle was significantly prolonged.

The investigations reported in the literature regarding the identity and existence of a luteotropic hormone (LTH) in domestic mammals

other than the rat and mouse are sometimes contradictory especially when considering the identity or even the role that such a hormone should play in the estrous cycle. However, hypophysectomy experiments strongly suggest that a luteotropic process of some sort does exist in most mammals. It has been proposed that a single luteotropic stimulus at ovulation in gilts and sows was sufficient to promote growth and functional activity of corpora lutea until about day 16 of the estrous cycle, but if hypophysectomy was performed early in the cycle, the life-span of the corpus luteum was not affected (49,131). Nalbandov (49) postulated further that no additional release of pituitary luteotropin occurred unless the gilt became pregnant. If pregnancy occurred then certain intra-uterine events caused a secondary release of luteotropin which then continued luteal life throughout pregnancy.

Inskeep _et al_. (146) showed that corpora lutea of various ages would regress at the same time in the ovary of the ewe. Different aged corpora lutea were developed by ovulating ewes in their existing luteal phase with sheep pituitary extracts. The regression of all corpora lutea at day 15 suggested that some factor predominated in determining the life-span of corpora lutea in sheep. Short (127) postulated that in the ewe a pituitary stimulus (luteotropin) was the "on" mechanism which tended to maintain the corpus luteum indefinitely. The "off" mechanism (luteolytic), which overrides the "on" stimulus, was produced in the uterus and thus actually controlled the length of the estrous cycle. Once the corpora lutea were formed in sheep, secretion of progesterone was automatic and the luteotropic hormone influenced the life but not the secretory activity of each corpus luteum.

A tremendous volume of literature has been published on many species, especially the rat, regarding factors which affect the life-

span and function of corpora lutea. Attempts to relate basic findings in different species usually ends in confusion. Thus it has become essential that investigations within species be conducted so that a quantal relationship of various hormonal parameters can be determined during successive stages of the estrous cycle. These evaluations are necessary before the luteotropic or luteolytic processes can be understood in any one species.

Luteal Phosphatase Activities During the Reproductive Cycle

Another parameter which may reflect the growth and regression of luteal tissue is the level of certain enzyme concentrations during the estrous cycle.

The distribution of alkaline phosphatase has been shown to be located in the theca interna cells of the porcine ovary during both the follicular and luteal phases of the estrous cycle (147). In more recent experiments, porcine corpora lutea had a mild alkaline phosphatase reaction when histochemical observations were made, and this activity appeared to increase slightly from estrus to 25 days after breeding (148). Goode (148) found that acid phosphatase activity was generally low in the ovary and did not appear to vary with the stage of the estrous cycle or pregnancy. O'Bannon _et al._ (149) reported, that in luteal tissue taken from 25 day pregnant gilts on different energy level diets, the average units (umoles of p-nitrophenol liberated/10 mg. tissue per hour) of alkaline and acid phosphatase activities were 9.61 and 1.73 units respectively. These results demonstrated that 25 day pregnant gilt corpora lutea have a dominant alkaline phosphatase component. Thus, if Corner's (147) observations were correct for porcine corpora lutea, there must be either a shift in phosphatase synthesis from the theca

lutein cells or an increase in the theca lutein cell component as pregnancy progresses in sows.

Stafford et al. (150) demonstrated that acid phosphatase in rat corpora lutea was always less than alkaline phosphatase during either diestrus, pregnancy or lactation. Acid phosphatase activity tended to increase in the latter stages of pregnancy as well as alkaline phosphatase activity. Alkaline phosphatase was found to dominate acid phosphatase during all stages of the estrous cycle or pregnancy.

Moss et al. (151) attempted to show by histochemical means that hydrolytic enzyme measurements may be used for early recognition of follicular atresia and luteal regression in rat ovaries. A rise in luteal acid phosphatase or aminopeptidase activities occurred at beginning involution in nonpregnant rats and in rats after parturition and lactation. This increased enzyme activity developed in lutein cells before gross regression of corpora lutea was detectable and the activity remained high until all recognizable parenchyma had disappeared. Involution of luteal tissue (physiological autolysis) was associated with the release of hydrolytic enzymes from lysosomes within the regressing tissue (152). However, these workers were unable to determine the precise time at which luteal regression began.

Taki et al. (153) have shown by histochemical studies of enzymatic patterns in human ovaries that alkaline phosphatase activity was markedly high in the theca lutein cells during the luteal phase of the menstrual cycle. This was thought to be related mainly to progesterone synthesis. Acid phosphatase was very low or absent in luteal cells during the active luteal phase of the cycle. It was postulated that both theca and granulosa lutein cells participate in progesterone synthesis during the normal menstrual cycle and during pregnancy.

Studies on the day-to-day fluctuations of total phosphatase activity in corpora lutea have not been reported. If the fluctuations were known and then related temporally to other changes, such as tissue and plasma FSH, LH, progesterone and estrogen, it may be possible to elucidate the luteotrophic and luteolytic mechanisms.

Luteal Progesterone Synthesis and Concentrations

Determination of progesterone concentrations in corpora lutea of the mammalian species is essential if the physiological functions of the ovary are to be related temporally with other endocrine changes during the estrous cycle. Studies related to the secretion and metabolism of progesterone have been hampered until recent years by the lack of chemical methods which were sufficiently sensitive and specific enough to quantitate the hormone in blood plasma and other tissue fluids. Many elaborate techniques have evolved during the past five years, such as thin-layer and gas-chromatography and isotope labeling techniques, which have made it possible to determine progesterone concentrations with greater precision (154,155,156).

A survey of the literature revealed that two types of corpora lutea exist in the several species studied. The existence of two types is based on steroidal content or the ability to synthesize certain steroid compounds (51). The first type of corpus luteum is that which can produce only progestins, such as progesterone and the epimeric 20β-hydroxy-Δ^4-pregnen-3-ones. This type is best exemplified by the bovine corpus luteum. Savard and Casey (50) found through _in vitro_ studies that the bovine corpus luteum was unable to form radioactive estrogen from acetate-1-^{14}C, testosterone-4-^{14}C, or progesterone-4-^{14}C precursors. It appeared that a deficiency in the aromatizing enzyme complex and 17-

hydroxylase existed in the bovine species (157). Likewise Short (127) indicated that the corpus luteum of the mare also belonged to this first type. However, the mare ovary does not produce estrogen due to a deficiency in its capacity to form androstenedione from 17-hydroxyprogesterone. Huang and Pearlman (158), using the superovulated rat, have studied the acetate-^{3}H metabolism of the rat corpora lutea *in vitro*. They observed that only radioactive progesterone and 20β-hydroxy-Δ^4-pregnen-3-one were produced. Duncan *et al*. (159) have reported the *in vitro* formation of progesterone from nonradioactive sources in the sow corpus luteum. Radioactive progesterone and 20β-hydroxy-Δ^4-pregnen-3-one were extracted from sow corpora lutea after incubation with acetate-1-^{14}C (51).

The second type of corpus luteum is that which produces both progestins and estrogens as well as 17-hydroxy-progesterone and androstenedione (51). The best representative of this group is the primate corpus luteum. Steroid synthesis *in vitro* has been studied using acetate-1-^{14}C as a precursor (158). The products from these *in vitro* incubations of primate corpora lutea were radioactive progesterone, Δ^5-pregnenolone, 20β-hydroxy-Δ^4pregnen-3-one, 17-hydroxy-progesterone, Δ^4-androstenedione, estradiol-17β and estrone. It appears that primate corpora lutea do not have the aromatizing enzyme complex deficiency as postulated for the other species.

Prior to the development and use of radioactive compounds for studying metabolic pathways, it was rather difficult to categorize the ovarian elements responsible for the actual synthesis of a particular steroid. However with the use of labeled acetate, it has been possible to demonstrate *de novo* synthesis of certain steroids and, with improved sensitive chemical techniques, to assess the presence of small amounts

of these steroids (155,156).

The possible mechanisms for regulation of the type and amount of steroid hormone produced by the ovary have been outlined by Ryan and Smith (160). The first theory considered was the two-cell theory for steroid hormone formation which suggested that the granulosa cell produced one type of hormone, the theca cell another, and that both types were necessary for estrogen production (127). Savard et al. (51) suggested that a third type of cell, the interstitial cell, may be involved in steroid synthesis as well. During proestrus and early estrus, Corner (24) observed that the theca interna of the procine ovary was highly vascularized, whereas the granulosa layers had no direct blood supply. When ovulation occurred, the granulosa cells enlarged and acquired a blood supply to form luteal tissue. Ryan and Smith (160) suggested that the vascularity of the granulosa in the follicle and its proximity to the theca interna required that steroids produced in the granulosa must tranverse the theca before entering the blood stream. The physical relationships of the granulosa and theca cells to the blood supply after ovulation suggested the changes in steroid synthesis seen at that time.

A second theory postulated for control of steroid synthesis by the ovary is that gonadotropins or other substances induce enzyme synthesis, enzyme activation or change of location within the cell (160). This accounts for the luteal phase trends in steroid production. For example, the rate of transformation of pregneolone to progesterone may be increased resulting in the accumulation of progesterone. The luteinization process may also enhance the accumulation of more substances which can be diverted into steroids (161). Savard et al. (51) suggested that conversion of acetate to cholesterol was the means of action of LH, and resulted in cholesterol substrate accumulation.

Other theories have been postulated to explain possible control mechanisms within the ovary (160). One is the alternate pathway theory which suggests that the major pathway of hormone formation during the follicular phase is via 17-hydroxypregnenolone and androstenedione. While in the luteal phase, the pathway was shown to be via progesterone to estradiol, depending on the species. Pituitary gonadotropin activity in the ovary dictates the pathway according to this theory. A second theory depends on the availability of NADPH as discussed by Savard _et al_. (51), and suggests that NADPH may be the limiting factor in hormone control mechanisms. And finally the steroids themselves may regulate the course of biosynthetic activity of the ovary by end product inhibition, enzyme activation, or enzyme induction (160). Although much is known about the gross changes of morphology and secretory activity in the ovary, little is known about control mechanisms for the fine hormonal balance apparently required in the reproductive cycle.

Duncan _et al_. (159) attempted to correlate corpora lutea size in swine with progesterone content at various stages of the estrous cycle. Corpora lutea increased in size from the 4th day (1.1 gm. luteal tissue per animal) to the 12th day (5.5 gm.) and then regressed to non-vascular corpora lutea by day 18 (1.4 gm.). Luteal progesterone followed a similar trend from the 4th day (23 ug.) to maximum levels at the 12th and 16th days (335 ug. and 211 ug. total) respectively. These investigators were unable to detect luteal tissue progesterone on day 18. Duncan _et al_. (85), in another series of experiments using pregnant gilts, found that corpora lutea had increased in size by day 16 (5.8 gm. luteal tissue/animal), and thereafter remained constant until gestation terminated. Total progesterone content on the other hand had undergone considerable change between days 16 (477 ug.) and day 102 (120 ug.) of

gestation.

Gomes *et al.* (154) determined the concentration of progesterone in ovarian venous effluent by cannulating the ovarian vein of the sow during the estrous cycle. Progesterone levels increased from an average of 0.03 ug./ml. of blood at estrus to a peak of 1.07 ug./ml. on days 10-12. The progesterone level decreased to 0.04 ug./ml. on days 19-21 of the cycle. This investigation indicated that progesterone levels in the ovarian venous effluent of the sow corresponded to the growth and regression of corpora lutea. Earlier, these workers reported a significant correlation between progesterone concentration in the bovine corpus luteum and ovarian venous plasma (162). Peak progesterone levels occurred between days 11 and 15. Short (163) estimated the progesterone secretion rates in ewes by cannulation of the ovarian vein. The same general trends reported for the sow were found except that peak progesterone levels occurred between days 8 and 16 of the cycle.

Uterine - Ovarian Relationships

Reproductive processes in many mammalian species indicate a physiological interdependence not only between the anterior pituitary and ovaries, but also the uterus. Loss of the uterus has been associated with profound changes in ovarian function in many species, but the factors underlying such changes have not been fully determined. Likewise, it is not known if the uterus secretes a hormone which modifies ovarian activity directly, or if the uterus is an afferent organ which exerts its control through the hypothalamus and anterior pituitary.

It has been known for at least 45 years that the uterus has an effect on the anatomical structure of corpora lutea in the guinea pig (164). However, renewed interest in this phenomena has existed only for

the past decade. Studies in many species have indicated that the uterus has a "lytic" action on the corpus luteum, although the mode of this action is unknown and its identity still remains obscure. Short (165) postulated that the uterine mechanism which apparently operates to some degree in most animal species to "shut off" the corpus luteum during the estrous cycle may prove to be the key to estrous cycle control in these species.

Three experimental approaches have been used to demonstrate the influence of the uterus on the life-span of the corpus luteum; (a) hysterectomy, (b) placing of foreign bodies inside the uterus, and (c) by administering agents thought to be luteolytic or antiluteolytic.

Hysterectomy and Luteal Function

Brown and Matthew (166), while observing the changes in estrogen and pregnanediol excretion in hysterectomized women, noted that cyclic ovarian function continued after hysterectomy. Hysterectomy in primates has not provided conclusive evidence that the uterus is essential in gonadal function (167). On the other hand, hysterectomy experiments in certain non-primate species, such as in cattle and swine have clearly demonstrated the apparent essential relationship between the uterus and cycling gonadal behavior. This procedure produces definite changes in luteal function in most of the domestic species tested and the degree with which the activity of the corpora lutea are affected varies somewhat between these species.

Ovarian cyclic activity was not affected in rabbits hysterectomized during estrus and before corpora lutea formation. However, if the uterus was removed after a sterile mating, corpora lutea life-span was extended from 16 to 30 days, and subsequent pseudopregnancies were also

lengthened (168).

Hysterectomy in the rat does not affect the normal cyclic life-span of corpora lutea unless performed on the pseudopregnant rat. The activity of the corpora lutea is prolonged from 12 days to 20 days in such rats (169). Melampy et al. (170) have found that the age of rat corpora lutea at hysterectomy in pseudopregnant animals determined the length of prolonged luteal activity. Hysterectomy on the 9th day gave the greatest prolongation of effect. It was also demonstrated that the quantity of uterine tissue removed at hysterectomy affected subsequent length of pseudopregnancy. Melampy et al. (170) also demonstrated that methods which eliminate the endometrium in rats, such as decidualization, produce an effect similar to hysterectomy on luteal function.

The length of the estrous cycle in the guinea pig is about 16 days, compared to 5 days in the rat. Corpora lutea of guinea pigs are functional during each estrous cycle and thus the stimulus of copulation is not essential for initiation of luteal function. Loeb (164) showed that hysterectomy markedly extended the luteal phase of the estrous cycle. Perry and Rowlands (169) reported that hysterectomy on day 5 after ovulation prolonged luteal function for 8 months, whereas hysterectomy on day 10 extended luteal activity for the approximate length of gestation (65-70 days). Butcher et al. (171) indicated that the reaction of the guinea pig corpus luteum to removal of the uterus changed on about day 6 after formation. This corresponded to the time of implantation in the guinea pig. It was also demonstrated that a functional relationship existed between corpora lutea and quantity of endometrium present. Thus if the endometrium was destroyed by chemical means, hysterectomy effects were observed based on luteal integrity and function.

The reproductive pattern of the ewe, cow and sow is generally

similar to that of the guinea pig, and hysterectomy effects are also similar.

If the uterus was removed from sheep anytime during the estrous cycle up to day 15, luteal function continued provided the luteal tissue was functional at the time of hysterectomy (145). Moor and Rowson (145) observed that if increasing proportions of endometrial tissue were removed in a series of ewes, the estrous cycles were lengthened accordingly. It appeared that much less endometrial tissue was necessary for normal luteal regression in the ewe than in the guinea pig, and the guinea pig in turn required less than the rat.

Short (127), following a review of reported evidence, postulated that the luteolytic effect of the uterus in sheep was dominant to the so-called pituitary luteotropic effect. The postulation was made that the pituitary "on" mechanism tended to maintain the corpus luteum indefinitely and that the uterine "off" mechanism actually controlled the length of the ovine estrous cycle. Denamur _et al_. (172) has reported that when the pituitary stalk of ewes was sectioned during the luteal phase of the estrous cycle that the corpora lutea regressed as normally expected. However, if hysterectomy was performed first and then the pituitary stalk was sectioned, the secretory life of the corpus luteum was prolonged. It was suggested therefore, that the uterus prevented the corpus luteum from responding to a luteotropic stimulus originating from the anterior pituitary. Deane _et al_. (173) studied certain structural and functional aspects of the regression of the corpus luteum in sheep using biochemical histological and electron microscope techniques. These investigators observed that alterations in the size and density of mitochondria of lutein cells on day 12 of the cycle were the first signs of luteal regression. The rapid decline in secretory activity seen on

day 15 was associated with a reduction Δ^5-3β-hydroxysteroid dehydrogenase and diaphorase activities as well as degenerative morphological changes. This abrupt cessation of secretion also suggested that a uterine factor predominated in regulating the estrous cycle in sheep.

Hysterectomy in the bovine has been shown to prolong the life of the corpus luteum for at least the equivalent of the gestation period (280 days) (174). It was observed that subtotal hysterectomy, whereby the cervix and varying proportions of the endometrium are spared, prolonged luteal function and the estrous cycle accordingly. However, Donaldson and Hansel (175) demonstrated that LH will prolong the life of bovine corpora lutea, even though the uterus is present, suggesting to them that uterine luteolytic effects are not as dominant in controlling the bovine estrous cycle as in the ovine. Armstrong and Black (176) reported that regressing bovine corpora lutea retained the ability to produce progesterone from exogenous pregnenolone. In their experiments, luteinizing hormone did not stimulate progesterone synthesis in regressing luteal tissue _in vitro_, but if NADP or G-6-P was added, the regressing luteal tissue synthesized progesterone. These investigators concluded that luteolysis in bovine corpora lutea was associated with the inability of luteal tissue to form pregnenlone.

Du Buisson _et al_. (177), Spies _et al_. (178) and Anderson _et al_. (179,180) reported that hysterectomy in the sow during the 16 days following the onset of estrus resulted in persistence of corpora lutea for 115 days. If the treatment was performed between days 16 and 18 of the estrous cycle, ovulation occurred as expected, but the newly formed corpora lutea persisted. Partial hysterectomy resulted in luteal regression and continuation of estrous cycles. This evidence clearly demonstrated the interdependence between uterine and luteal function in

the porcine. Also the proportion of uterus which remained after partial hysterectomy altered the estrous cycle by lengthening it until one-fourth of one uterine horn remained. One-fourth of the total uterine tissue was the minimal quantity necessary for bilateral luteal regression in the pig (179).

It has been reported that autotransplants of uterine tissue to other areas of the body in the pig resulted in continuation of normal estrous cycles (180). These results suggested that the luteolytic activity of the uterus in swine was able to operate without a major nerve supply. However the blood and lymph supply were still present.

The existence of a local mechanism for unilateral regression of corpora lutea has been observed in partially hysterectomized pigs but the mode of this action has not been revealed. One-sixth of one uterine horn caused regression of the ipsilateral corpora lutea, but the contralateral corpora lutea persisted as in the complete hysterectomy experiments (181). Anderson (182) postulated that a local mechanism altered neural activity and/or the blood and lymph supply to the ipsilateral ovary and uterine horn at certain stages of the cycle. However Oxenreider _et al_. (183), in a detailed study of the anatomy of the vascular system of the uteri and ovaries of sows, found no anatomical evidence of a neural or circulatory system which would account for this local utero-ovarian mechanism in swine. Short _et al_. (163) reported that one of the easiest way to inhibit secretion of progesterone by the corpus luteum of sheep was to interfere in some way with its blood supply. Anoxia appeared to have a profound depressant effect on steroid synthesis. Thus the suggestion was made that ovarian vascular control mechanisms may be concerned in the process of luteolysis.

It has been reported that the presence of a nongravid uterine horn

in the pig usually resulted in early embryonic death in the opposite horn. Pregnancy continued if the nongravid horn was removed before day 14, whereas pregnancy failed if it was removed after day 16. Pregnancy continued in the gravid horn if one-eighth of the non-gravid horn remained intact, but luteal regression occurred on the side of the retained uterine fragment (181,184). Anderson et al. (179) observed that pregnancy failed in pigs with a gravid left horn and seven-eights of a non-gravid right horn. However, unilateral pregnancy was maintained 25 days with daily injections of progesterone in animals with equal amounts of the right nongravid horn. Luteal progesterone content was significantly reduced in the ovary adjacent to the nongravid horn, whereas it increased significantly in the horn adjacent to the gravid horn between days 13 and 15 of pregnancy. A compensatory response occurred in corpora lutea adjacent to the gravid horn since the corpora lutea adjacent to the nongravid horn had regressed. He also reported that the 16th day after estrus in swine represented a critical period for either persistence or regression of corpora lutea.

It has been suggested that the luteolytic action of the uterus in swine is clearly dominant to the so called pituitary luteotropic stimulus (181,182). However, Neill and Day (185) found that corpora lutea were induced to develop in luteinized ovaries of gilts by gonadotropin treatment and grow along with the normally existant corpora lutea. These newly formed corpora survive for a certain length of time despite regression of the pre-existing set. If hysterectomy was performed early in the luteal phase then both old and new corpora lutea persisted. Spontaneously formed and induced corpora lutea in the ewe regressed simultaneously (146).

Moor and Rowson (186) demonstrated the local mechanism of

luteolysis in sheep. In a series of experiments on unilaterally ovariectomized ewes in which the adjacent or opposite uterine horns were removed, variations in estrous cycle lengths were observed. If the uterus and ovary were adjacent the estrous cycle length was normal, but if the uterine horn adjacent to the intact ovary was removed the cycles were prolonged. However these cycles were not as prolonged as in the bilateral hysterectomy experiments, suggesting to these investigators that the uterine luteolytic effect was first local and then became general as the intensity increased.

It is evident then that the life-span of corpora lutea is closely associated with changes in the uterus. Also it has been shown that the life-span of corpora lutea is limited in the presence of the endometrium in domestic animals. Removal of the endometrial influence by hysterectomy, chemical means, deciduomata formation (rat) or by pregnancy favors the secretion of progesterone for a much longer period of time. Species differences in the mechanism controlling luteal function may only be due to differences in the intensity of action of certain components of that mechanism.

Intrauterine Foreign Bodies and Luteal Function

Another line of evidence for uterine involvement in luteal function was first reported by Moore and Nalbandov (187,188). These investigators showed that when 8 mm. diameter glass beads were introduced into one horn of the ovine uterus on day 3 of the estrous cycle, early regression of corpora lutea and shortening of the estrous cycle resulted (16.3 days to 11.5 days). Further, if the beads were placed in the uterus on day 8 of the cycle, the estrous cycle was lengthened (16.3 days to 21.6 days). Size of the beads seemed to influence the response

suggesting that a neural mechanism was involved. These findings have not been substantiated in full by other workers. However Inskeep et al. (189) observed that distension of the ovine uterus on day 3 shortened the estrous cycle, but placement of beads on day 8 failed to extend the cycle. Distension of the uterus appears to modify the periodicity of the estrous cycle in the cow (190). Donovan and Traczky (191) found that when 2 glass beads were placed in each uterine horn of the guinea pig, the estrous cycle was shortened by 3-4 days. This response was lost by the 9th or 10th day of the cycle. Moore (192) found that in the guinea pig, when small beads were sutured into one uterine horn by day 3, the cycle was shortened to 11.7 days, but if the operation was performed on day 8, the cycle was prolonged to 22.4 days. Bland and Donovan (193) have reported that uterine distension with glass beads altered luteal function in guinea pigs depending on whether the beads were unilaterally or bilaterally placed. Two beads in each horn shortened the cycle by 3 days when placed during the first 4 days of the cycle, whereas one bead in each horn shortened the cycle by 1.75 days. Four beads in the right horn shortened the cycle by 1.7 days while 2 beads do not alter the cycle. This further demonstrated local uterine effects on luteal function.

Hansel and Wagner (190) demonstrated that dilatation of the bovine uterus with a rubber catheter between days 1 and 7 shortened the estrous cycle from 20 days to 12-13 days. Anderson et al. (180) also observed that suturing plastic or vitalluim cylinders into the uterine horns shortened the estrous cycle in most cases. Yamauchi et al. (194) studied the effects on the estrous cycle of the cow when a viscous gel-like substance was injected into the uterus. Treatment in the early luteal phase shortened estrous cycle length to 12.5 days, whereas late

luteal phase treatment prolonged the cycle to 25.8 days. Treatment during proestrus, metestrus and diestrus (about day 12) did not alter cycle length in 85% of the animals involved. It was not determined if the bovine uterus distended by gel injection directly affected luteal function or if a neurogenic stimulus from the distended uterus modified the secretion of pituitary gonadotropins.

In most cases reported intra-uterine contraceptive devices do not appear to alter the menstrual cycle significantly in women (167). Likewise Anderson et al. (174) and Rathmacher et al. (195) were unable to alter estrous cycle length in swine by placing foreign objects of different sizes in the uterus.

Embryo Influence on Luteal Function

Moor and Rowson (186) have shown that transfer of embryos to non-pregnant ewes up to the 12th day after estrus consistently results in pregnancy. However embryos transferred on days 13 or 14 had little or no effect on luteal life-span even though the embryos were viable. Apparently embryos transferred at the proper time were able to exert a luteotropic effect on the luteal tissue of the recipients. Conversely, if the embryo was removed from the donor ewe before day 12 then the donor animal was never aware of its pregnancy, but if the embryos were removed on days 13, 14 or 15 the estrous cycle was prolonged due to continued luteal function.

Short (165) suggested that the embryo exerts an "antiluteolytic" action rather than a "luteotropic" one. He reported that if hypophysectomy was performed on day 30 of pregnancy in sheep then luteal regression and abortion followed. However, if hypophysectomy was performed on day 60 then no immediate effect on luteal function was noted. A

placental luteotropin may be involved.

Rowson and Moor (196) reported on the influence of embryonic tissue on luteal function when infused into the uterus. Daily intra-uterine infusions of 14 and 15 day sheep embryo homogenates prepared from frozen tissue significantly prolonged the estrous cycle in recipient ewes. If 14 day pig embryos were used no difference was observed in estrous cycle length. Embryos from 25 day pregnant ewes did not alter the estrous cycle. Thus stage of embryo development and species specificity appeared to be factors in the anti-luteolytic mechanism. The later mechanism may not be identical in all species.

The ovulation rate in swine is usually between 12 and 18 ova unevenly distributed between the two ovaries. However embryonic migration occurs after fertilization and the blastocysts are evenly distributed within the uterus by the 14th day of pregnancy (24,197). Perry and Rowlands (198) showed that blastocysts elongate and occupy nearly the entire length of the uterus by day 14. Polge et al. (199) observed that more than four embryos were required to establish and maintain pregnancy in the sow. One embryo significantly prolonged the estrous cycle by 6 days. Four embryos or less were unable to completely overcome the luteolytic influence normally exerted by the non-pregnant uterus.

Du Buisson and Rombauts (200) observed that removal of some fetuses with their corresponding uterine segment between days 40 and 80 of gestation terminated pregnancy in about 50% of the gilts. However, removal of fetuses alone, terminated pregnancy in 80% of the gilts. It appeared that in the latter stages of pregnancy, the ratio of embryonic tissue to endometrial surface area was a factor in the maintenance of pregnancy.

Dhindsa and Dzuik (201) observed 169 gilts which were used to investigate the influence of killing or removing embryos in one uterine

horn between days 4 and 50 of gestation. Control gilts were treated similarly except that the embryos were not killed or removed. No difference was observed between treated and control gilts in the proportion which remained pregnant when treated between days 12 and 50. None of those treated between days 4 and 10 remained pregnant. They concluded that embryos must be present in both uterine horns between days 10 and 12 for continuation of pregnancy, while embryos need not be present in both horns after day 12 for pregnancy to continue in swine.

Phosphatase Activities in Endometrial Tissue

Uterine endometrial and myometrial physiology have been found to be regulated by gonadal hormones. Talmage et al. (202) studied estradiol influence on alkaline phosphatase activity in the reproductive tract of the rat and reported that estrogen caused a marked increase in alkaline phosphatase activity of secretory epithelium and in the circular musculature. Giering and Zarrow (203) demonstrated, that initial doses of estradiol markedly raised the level of alkaline phosphatase activity and caused significant uterine growth. Chronic doses of estradiol caused a decrease in alkaline phosphatase levels and atypical endometrial growth. High initial doses of progesterone caused a transient rise in uterine alkaline phosphatase activity and some uterine growth, but chronic doses caused uterine atrophy and decreased alkaline phosphatase activity. Alkaline phosphatase seemed to possess a duel role in uterine physiology associated with growth and secretory processes of the endometrium as well as with the contractile activity of the myometrium. The changes in phosphatase levels reflected changes in general uterine metabolism.

Manning et al. (204) recently reported on histochemical studies of

endometrial acid and alkaline phosphatases in ovariectomized Rhesus monkeys treated with different combinations of estrogen, progesterone and relaxin. Following estrogen treatment, profound alkaline phosphatase reactions occurred in the surface and glandular epithelium of the endometrium. Progesterone combined with estrogen resulted in a marked reduction of alkaline phosphatase activity. These changes were similar to those described for the luteal phase of the menstrual cycle of women (205).

Manning _et al_. (204) observed that estrogen in some way potentiates the synthesis of acid phosphatase in all areas of endometrial tissue. This effect was reversed by progesterone in surface epithelia and was markedly decreased in glandular epithelium when relaxin was added to the treatments. This contrasted with McKay's (205) work which showed that increased acid phosphatase activity occurred during the luteal phase of the menstrual cycle in women. Manning _et al_. (204) also noted that addition of progesterone or progesterone-relaxin combinations to estrogen treatments increased the number of acid phosphatase positive stromal cells, especially in the surface epithelium.

Leathem (206) studied the 72 hour response of the immature mouse uterus to estradiol, testosterone and progesterone treatments. Alkaline phosphatase and β-glucuronidase were evaluated. Estrodiol markedly increased alkaline phosphatase activity while testosterone and progesterone had no effect.

Functional studies of uterine RNA by Mansour _et al_. (207) demonstrated that RNA isolated from the estrogen activated uterus can markedly stimulate biosynthesis of alkaline phosphatase in the castrated mouse uterus. This provided evidence that uterine alkaline phosphatase was directly related to estrogenic stimulation.

Manning et al. (208) showed that alkaline phosphatase activity in the uterine horn of the pregnant rat increased from the 5th to the 8th day of pregnancy, whereas alkaline phosphatase activity in the non-gravid horn of the same rat remained low. Alkaline phosphatase activity was increased in the gravid horn at the implantation sites. Enzyme activity was apparently dependent upon the interaction between blastocysts and the hormonally conditioned uterus, rather than upon hormonal stimulation alone.

Goode et al. (209) indicated that both acid and alkaline phosphatase activities in the endometrium of gilts were significantly different during various reproductive stages. The average units (activity per mg. fresh tissue, per one-half hour) of alkaline and acid phosphatase activity, respectively were; (a) 8 days after estrus, 7.0 and 1.5; (b) 18-19 days after estrus, 1.1 and 1.8; (c) during estrus, 3.4 and 1.0; (d) 3 days after breeding, 9.3 and 1.1; (e) 8 days after breeding, 6.7 and 1.6; (f) 12 days after breeding, 1.9 and 2.6; (g) 25 days after breeding, 0.5 and 5.7. These two types of phosphatase activity appeared to be inversely related to the endometrium.

Morrissette et al. (210) demonstrated that uterine acid phosphatase was much greater than alkaline phosphatase activity in nonpregnant gilts with cystic ovaries.

Goode et al. (148) reported on the distribution and levels of acid and alkaline phosphatase activities in the endometrium of pregnant gilts. Endometrial acid phosphatase activity increased from 1.03 units/10 mg. tissue per hour at estrus to 5.71 units at 25 days post-breeding.

The endocrine mechanism of formation and function of the corpus luteum has come to occupy the major endeavors of a number of research workers in reproductive physiology and endocrinology. For herein lies

the basic control of the cycling phenomena in female reproduction of mammals. Most of the literature reviewed here has provided some valuable insights on various aspects of the porcine estrous cycle, though many theories have been gleaned from data in rats and other species. More and more evidence indicates that each species has its own mechanisms of estrous cycle control which may or may not be applied to another species. Therefore, the tendency in reproductive physiology research should be of necessity directed toward the investigation of basic facts of species-specific estrous cycles. Very little research has been directed toward the study of temporal relationships between anterior pituitary and plasma gonadotropin levels, and luteal growth, maintenance and function, and uterine influence on these, during successive periods of an estrous cycle in any one species. A detailed study of selected correlated parameters in a single species should provide information on the specific mechanisms of estrous control. It was with this idea in mind that the following study was designed.

CHAPTER III

MATERIALS AND METHODS

General

Treatment of Animals: Eighty-four crossbred, nulliparous gilts (Duroc, Hampshire and Yorkshire breeds), 6 to 7 months of age were checked daily for estrus behavior with vasectomized boars. Each gilt was observed throughout one estrous cycle before assignment to a specific day of a predetermined 20-day cycle and eventual slaughter. Three vasectomized boars were rotated daily for a one hour period (9-10 a.m.) among three pens of 20 gilts each, and one pen of 24 gilts. The first day of the estrous cycle was defined as the day in which each gilt would first mate with a vasectomized boar. Each vasectomized boar was allowed to search out those gilts in each pen showing any degree of estrous behavior. Those gilts were marked and then each boar was permitted to mate with at least one or two, depending on the number of gilts (day 1) that were in definite estrus. Day 2 or day 3 gilts were not bred again. The number of gilts in estrous (day 1) ranged from 0 to 5 each day. Three to 5 gilts were assigned to each day of a predetermined 20 day estrous cycle. Attempts were made to select gilts for day 21, but most came into estrus 1 or 2 days early, therefore the predetermined cycle was limited to 20 days.

Assigned gilts were slaughtered at a local abbattoir[1] in groups of

[1]Ralph's Packing Co., Perkins, Oklahoma.

5 per day during each 5 day period of the week. Each group was slaughtered between 12:30 and 1:00 PM, and all specimens were obtained within 30 minutes after slaughter.

Animals were initially assigned so that the above schedule could be followed. Those gilts which could not be assigned according to this regime were allowed to cycle again and then assigned.

Injuries from shipment of gilts, abnormally short estrous cycles, and the appearance of cystic ovaries at slaughter eliminated 5 of the original 84 gilts. The remaining 79 gilts were considered normal based on estrus behavior and inspection at slaughter.

Collection and preservation of specimens: At slaughter approximately 500 to 800 ml. of citrated blood were collected from each animal by severing the anterior vena cava. Each 500 ml. of citrated blood containing 140 ml. of sterile concentrated ACD[2] along with one ml. of penicillin-streptomycin[3]. The blood was gently agitated soon after collection with the ACD solution to prevent clotting, and then packed in wet ice (0°C.) for transport to the laboratory. The citrated blood from each gilt was centrifuged at 11,000g, 7°C for 20 minutes in an International Model HR-1 refrigerated centrifuge. The recovered plasma was then placed in sterile quart milk cartons, sealed and stored at -30°C.

The entire uterus and both ovaries were removed from the carcass of each gilt within 15 minutes after slaughter. The ovaries were immediately removed and placed in a plastic home-freezer bag. A transverse section of uterine tissue measuring approximately 15 cm. in length was removed from the posterior one-third of the right horn, trimmed and

[2]Sodium citrate 2.2gm.; citric acid 0.8gm.; dextrose 2.45gm., per 100 milliliters of solution.

[3]200,000 units penicillium and 0.25 gm. streptomycin per milliliter.

placed in a plastic bag. These specimens were then placed in a paper milk carton and packed in dry ice (-78.5°C) for quick-freezing and transport to the laboratory. The samples remained on dry ice for another 24 hours and were then stored at -30°C.

The entire pituitary gland was removed 30 minutes after slaughter, trimmed, and placed in 50 ml. of acetone for defatting. Twenty-four hours later the acetone solution was removed and 50 ml. of fresh acetone added. Seventy-two hours after slaughter, the second solution of acetone was removed and subsequently the posterior pituitary gland was separated from the anterior pituitary gland. The anterior portion was placed in a desiccating jar over phosphorus pentoxide (P_2O_5) and dried for another 48 hours under vacuum. After drying, the anterior pituitary glands were weighed to the nearest tenth of a mg. on an analytical balance. Each gland was stored in a sealed container at -30°C.

Part I

<u>Preparation and Photography of Specimens</u>: The ovaries from the 79 gilts described above were partially thawed and trimmed of all periovarian structures which were not removed at slaughter. This procedure was carried out in a cold room at 5°C. In turn as each pair of ovaries was thawed and trimmed, they were photographed.

An Exakta single reflex camera with a Carl Zeiss Jena lens (F 2.8), polaroid filter, and Kodachrome II type A professional film was used for photographing the specimens. Photographs of each pair of ovaries were taken over an opal glass-fluoresent light background. No distinction was made between right and left ovaries in the photographs. Photographic magnification was .85x.

From the group of 79 pairs of ovaries, a selected set of 20 were

chosen to represent the characteristic ovarian morphological changes observed during the predetermined 20 day cycle.

Part II

Preparation of Specimens for Chemical Assay: After the partially-thawed ovaries from each of the 79 gilts were photographed in Part I the corpora lutea were counted and then extirpated. The extraovarian tissue and corpora lutea were weighed separately on an analytical balance to the nearest tenth of a mg. Subsequently, based on total luteal weight of both ovaries, enough cold 0.9% sterile NaCl was added to a known weight of luteal tissue to make a 20% homogenate. The luteal homogenate was prepared with a Potter-Elvehjem homogenizer. Each homogenate preparation was then refrozen at -78.5°C. and stored at -30°C. until analyzed for phosphatase enzyme activity.

The transverse section of uterus taken at slaughter was allowed to thaw slightly and the endometrium was separated from the remainder of the uterine tissue with a pair of hemostatic forceps and a single edge razor blade. The wet endometrial tissue scrapings were weighed to the nearest tenth of a mg. and 20% homogenates were prepared in cold 0.9% sterile NaCl with a Potter-Elvehjem homogenizer. The resulting homogenate from each gilt was refrozen immediately at -78.5°C. and 24 hours later placed in storage at -30°C.

Acid and alkaline phosphatase activities of both luteal and endometrial homogenates were analyzed according to the methods of Bessey et al. (211) and Andersch and Szczypinski (212) with modifications outlined in Sigma Technical Bulletin #104 (213). The original 20% crude homogenates of both luteal and endometrial preparations were assayed at a dilution which gave optimum readings on a Model 14 Coleman

Spectrophotometer. Most of these homogenates were assayed at 1% and 2.5% dilutions respectively, based on the original wet weights. All tissue homogenate preparations and assays were conducted in a cold room at 5°C.

Acid and Alkaline Phosphatase Assay: Tissue enzyme activities were expressed as units of enzyme activity per mg. of soluble protein. One unit (Sigma) of enzyme activity was defined as the amount of phosphatase which would liberate 1 umole (0.1391 mg.) of p-nitrophenol from p-nitrophenol phosphate per hour under the conditions specified in Sigma Bulletin #104 (214). A standard curve was prepared using selected dilutions of p-nitrophenol standard solution according to the procedure outlined in the bulletin. Optical densities were determined on a Model 14 Coleman Spectrophotometer at a wave length of 410 mu. The optical density of each unknown sample was translated from the standard curve as the phosphatase activity per ml. of homogenate. Total protein concentration per ml. of homogenate was determined by the trichloracetic acid precipitation method outlined by Stadtman et al. (214) using bovine serum albumin as the standard protein. Enzyme activities in Sigma units per mg. of soluble protein were calculated for both acid and alkaline phosphatase in each homogenate sample.

The individual acid phosphatase or alkaline phosphatase activity data for each gilt was obtained and averaged for each day of the estrous cycle. Values for each two consecutive days, starting at day 1, were combined and averaged as shown in tables I and II. Analysis of variance, standard deviation statistical techniques were applied to test for difference between days and variation of the mean values, respectively, according to the basic techniques of Ostle (215). An analysis of variance summary was prepared as shown in table III.

A ratio of the acid phosphatase activity to the alkaline phosphatase activity, for both the corpora lutea and the endometrium was calculated for each of the 10 composite periods of the estrous cycle.

Part III

Preparation of Stored Plasma and Pituitary Specimens for Bio-assay:

The frozen citrated plasma samples from those gilts assigned to a specific day of a predetermined 20-day estrous cycle were thawed and pooled. Twenty pooled plasma samples from 72 gilts were prepared in this experiment along with the corresponding pooled samples of corpora lutea and anterior pituitary glands. Seven of the original 79 gilts were not used in this study, since the investigator was unable to obtain sufficient plasma at slaughter.

Each pooled plasma sample, representing a mean of 3.6 gilts, exceeded 2 liters in quantity. Five hundred to 700 ml. of each pooled plasma sample were shell frozen in 4 (1000 ml.) round bottomed flasks and then lyophilized. Each 8-11 grams of lyophilized plasma was equivalent to 100 ml. of pooled citrated plasma. Another 600 to 900 ml. of each plasma sample were concentrated for luteinizing hormone (LH) activity according to a method outlined by Anderson and McShan (118). The purified precipitates obtained in the last step of this method were subsequently dissolved in distilled water, shell frozen and lyophilized. The dried fractions were weighed, placed in glass tubes, and then sealed and refrigerated at -30°C. until assayed for LH activity. Each mg. of the lyophilized material was equivalent to 2.5-5.0 ml. of pooled plasma. The crude and purified lyophilized samples were stored at -30°C. until

assayed for FSH and LH activity, respectively.

The defatted-dessicated anterior pituitary glands of each group of gilts assigned to a particular day of the estrous cycle, were pooled and homogenized in cold 0.9% NaCl with a Potter-Elvehjem homogenizer. The volume of cold 0.9% NaCl used was adjusted so that the final homogenate concentration was 10 mg. of dry anterior pituitary gland per ml. of homogenate. Aliquotes of the homogenate were then assayed for FSH and LH activity, respectively.

Follicle Stimulating Hormone Assay: The HCG-Augmentation assay of Steelman and Pohley (98) as modified by Parlow and Reichert (216) was used. For 3 days, 24 day old female rats of the Holtzman strain[4] were injected subcutaneously twice daily (8 AM and 5 PM) with various test preparations suspended in 0.9% NaCl to which 20 IU of HCG[5] had been added *in vitro*. On the morning of the 4th day all rats were euthanized with ether. The ovaries were removed, freed of adherent fat and connective tissue, and weighed to the nearest tenth of a mg. on an analytical balance.

All FSH assays were balanced and of 5-point design in which 2 levels of a standard FSH preparation, 2 levels of a test preparation, and one blank preparation were tested. Six or 7 animals were assigned at each dose level. The interval between successive dose levels was 2-fold.

The standard FSH preparations[6] used were as follows; (a) NIH-FSH-P_1 was used at dose levels of 150 and 300 ug. per rat respectively in the

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[4]Holtzman Company, Madison 5, Wisconsin.

[5]Human Chorionic Gonadotropin, Jen-Sal Company, Kansas City, Missouri.

[6]Granted by the Endocrinology Study Section, National Institutes of Health, Bethesda, Maryland.

anterior pituitary gland assays, (b) NIH-FSH-S_1 was used at 160 and 320 ug. levels per rat respectively in the purified plasma LH assays, (c) all blank preparations as well as standard and test preparations contained 0.9% NaCl as the carrier vehicle plus 20 IU HCG.

The test preparation levels for the pituitary homogenates were prepared so that each rat in the low or high dose level received 5 mg. or 10 mg., of dry pituitary equivalent respectively, over a 3 day period. The lyophilized plasma extracts were prepared so that 5 ml. or 10 ml. of plasma equivalents were given to each test rat at each respective dose level, over the 3 day injection period. The volume of each injection was 0.5 ml.

Mean relative potency and 95% fiducial limits of the pituitary gland and plasma extracts were evaluated by the slope-ratio method outlined in Finney (217). The validity of each assay and the heterogeneity of variances were calculated with the aid of the 7040 IBM computer[7].

Luteinizing Hormone Assay: The ovarian ascorbic acid depletion (OAAD) assay of Parlow (91) was used to assay LH activity in both anterior pituitary gland homogenates and extracted plasma. Immature 24 day-old female rats of the Holtzman strain were used. These rats were prepared for bioassay by a single subcutaneous injection of 75 IU PMS[8], followed 60 $\pm$ 5 hours later by a single subcutaneous injection of 50 IU HCG. Pregnant mare's serum was administered subcutaneously at 5 PM of the day following receipt of the rats, whereas the injection of HCG was given subcutaneously at 8 AM on the 3rd day following PMS treatment. The animals became pseudopregnant and were ready to be used for the

[7]Department of Statistics, Oklahoma State University, Stillwater, Oklahoma.

[8]Pregnant Mare's Serum, Haver-Lockhart, Kansas City, Missouri.

assay of LH activity 7 days after the HCG treatment.

At the time of assay the rats were lightly anesthetized in a large jar containing concentrated ether vapors. The material to be assayed for LH activity, previously dissolved to make 1.0 ml. of saline solution per 100 grams of rat body weight, and was injected intravenously via the tail vein with a 27 gauge half-inch needle. All injections of pituitary homogenates, or purified plasma extracts, were made uniformly over a 15-20 second period.

Four hours ± 10 minutes after the intravenous injection of the test samples each rat was euthanized with ether and the right ovary was removed immediately. The ovary was quickly freed of fat on a moistened paper towel and briefly blotted on dry filter paper to remove adherent blood or moisture. The ovary was then weighed to the nearest tenth of a mg. on an analytical balance and then transferred to a glass Ten Broeck tissue grinder[9]. The ovary was crushed against the side of the grinder and 10 ml. of 2.5% metaphosphoric acid was added before the tissue was homogenized. The homogenate thus formed was filtered through Watman #40 filter paper into test tubes which were precooled in an ice-bath. These filtrates were frozen and stored at -30°C. until analyzed for ascorbic acid content.

The clear metaphosphoric acid ovarian filtrates were analyzed for ascorbic acid according to the method of Mindlin and Butler (218) as modified by Munson[10]. One hundred mg. of ascorbic acid (U.S.P. reference standard) was dissolved in 2.5% metaphosphoric acid and made up to a volume of 100 ml. Standard solutions of ascorbic acid ranging in concentration from 2 to 6 ug. per ml. were prepared from this stock

[9]Corning pyrex #7727.
[10]Dr. A. F. Parlow (Personal communication).

solution. A standard curve was plotted for these standards using a Model 14 Coleman spectrophotometer. The standard curves were used to evaluate the quantity of ascorbic acid in each rat ovary. The substrate for all ascorbic acid assays was 2,6-dichloro-indophenol buffered with 4.53% sodium acetate to ph 7.0. Five milliliters of 2,6-dichloro-indophenol-acetate solution was added to each of a series of clean test tubes, and 2.5 ml. of each ovarian filtrate plus 2.5 ml. of 2.5% meta-phosphoric acid was added to another series of clean tubes. A blank was prepared by adding 5 ml. of 2.5% metaphosphoric acid to one of the latter tubes. To each indophenol-acetate solution, a corresponding 2.5% metaphosphoric solution of ovarian ascorbic acid filtrate or standard was added, mixed and read in a Model 14 Coleman spectrophotometer at 515 mu. The optical density was determined in not less than 20 seconds and not more than 45 seconds after mixing the two solutions. This procedure was followed for each test sample. Various alterations in the proportion of ovarian filtrate added were necessary in some samples in order to evaluate them within the range of the standard curve.

The total quantity of ascorbic acid in the ovarian filtrate was obtained by multiplying the value obtained from the ascorbic acid standard curve by the total volume of metaphosphoric acid solution in the homogenate. Those ovarian filtrates which were further diluted for analysis were also multiplied by a dilution factor. The final results were expressed as micrograms of ascorbic acid per 100 mg. of ovary.

All LH assays were a balanced 4-point design, with 2 levels of a standard LH preparation, and 2 levels of a test LH preparation evaluated in each assay. Five assay rats were assigned to each dose level. The interval between successive dose levels was 4-fold.

The standard LH preparations[11] used in these assays were NIH-LH-B_4 and NIH-LH-S_{11}, both of which were used at dose levels of 0.5 and 2.0 ug. per rat, respectively.

Preparations of the anterior pituitary gland assayed contained 0.5 mg. and 2.0 mg. of dry weight per 100 grams of assay rat for the low and high dose levels, respectively. The concentrated plasma LH extracts, were divided so that each assay rat received the equivalent of 15 ml. of pooled whole plasma for the low dose, and 60 ml. for the high dose per 100 grams of assay rat.

A parallel-line statistical method outlined by Finney (217) for multiple assays was used to determine the relative potency and fiducial limits. Statistical tests such as the analysis of variance, variance heterogeneity and parallelism were calculated with the aid of the 7040 IBM Computer.

Part IV

Preparation of Specimens for Chemical Assay: The remainder of the luteal homogenates prepared in part II were assayed for progesterone concentration. The luteal homogenates from each of the 79 gilts were grouped according to the assigned day of a 20 day predetermined estrous cycle, and then pooled. Thus 20 pooled luteal tissue homogenate samples, averaging 3.95 gilts per sample, were analyzed for total progesterone content by a chemical assay procedure.

Luteal Progesterone Assay: The 20% pooled luteal homogenates were extracted with hot acetone (CP grade) after 22 x 10^{-3}uC of 7α-^{3}H-pro-

[11]Granted by the Endocrinology Study Section, National Institutes of Health, Bethesda, Maryland.

gesterone[12] was added to estimate recoveries. The samples were analyzed for progesterone content based on the methods of Ryan and Smith (160), and Hellig and Savard (219), as modified by Cardeilhac _et al._ (220). The following general sequence was followed in the isolation and quantitation of progesterone in the luteal tissue homogenates; (a) Each 20% pooled homogenate, which contained a tritium labeled progesterone marker, was extracted with 15 volumes of hot acetate (54-60°C.) and then suction-filtered through a Buchner funnel using #40 Whatman filter paper; (b) The filtrate was evaporated to dryness using a Buchi Rotavapor[13] and a 45°C. water bath; (c) Five milliliters of water and 0.25 ml. of 10% NaOH were added to the dried material, followed by extraction 3 times with 10 ml. diethyl ether; (d) The ether extract was then washed 3 times with 2 ml. water followed by evaporation of the ether under nitrogen; (e) The dry material was reconstituted in 2 ml. of 90% aqueous methanol, and extracted 3 times with 2 ml. portions of legroin; (f) The legroin was discarded and the aqueous methanol phase was evaporated to an aqueous residue under nitrogen; (g) Water was added to the aqueous residue to a volume of 0.5 ml., and this residue was extracted 3 times with 1.5 ml., of diethyl ether. The ether was evaporated and the dry residue resuspended in 0.1 ml. of tetrahydrofuran (THF); (h) Fifty microliters of the THF solution, along with 10 ml. of standard progesterone[14] (1 mg./ml.) were spotted separately on silica gel[15] thin layer plates which had been activated by heating at 110°C. for 60

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[12]1300 mc/m mole, obtained from Nuclear-Chicago Corporation, Des Plaines, Illinois

[13]Distributed by Pinco Instrument Company, Greenville, Illinois.

[14]Sigma Chemical Company, St. Louis, Missouri.

[15]MN-Silica Gel G-HR/UV_{254} 30 gms. plus 1 ml. 95% Ethanol plus 59 ml. tap water. Spread 1/4 mm. thick over clean glass plates. Silica Gel G distributed by Brinkman Instruments, Westbury, N. Y.

minutes. The thin-layer plates were then chromatographed in a chloroform-methanol solvent (99:1). Some samples required a second thin-layer purification step in which another chloroform-methanol ratio (95:5) was used; (i) After chromatography, silica gel was removed from those bands outlined on the thin-layer plates which corresponded to the standard progesterone bands as determined by ultra-violet fluorescence. These silica-gel samples were then extracted 3 times with a small volume of diethyl ether; (j) The ether extracts were evaporated to dryness, and reconstituted in 1.0 ml. of diethyl ether. A tenth milliliter portion of each ether extract was transferred to a vial containing 10 ml. of scintillation fluid[16] for counting and the per cent recovery of 7a-^{3}H-progesterone determined. The remaining nine-tenths of each solution was again evaporated to dryness under nitrogen; (k) The dried material was reconstituted in .05 ml. of THF. An aliquot of 2 to 5 ul. was injected into a Barber-Coleman gas chromatograph[17] using a 1% XE commercially packed column as the stationary phase and a hydrogen flame ionization detector. Injector and column bath temperature settings were 250°C and 220°C respectively. A nitrogen carrier gas glow rate of 48 ml./min. was used. The standard progesterone retention time was approximately 11 minutes. The detector temperature was 230°C and the attenuation settings were generally at 100. Various quantities of standard progesterone (1 mg./ml.) were injected and retention time measured. Peak areas were measured by planimetry. A standard curve was drawn and from this curve the test sample peak areas were evaluated. (l) Liquid scintillation spectrometry was accomplished with a Tri-Carb[18] scintillation counter. The recovery of tritium labeled progesterone was determined by counting at a

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[16]4 gms. PPO and 0.3 gms. POPOP-Benzene per liter.
[17]Selecta-5000, Barber-Coleman Co., Rockford, Illinois.
[18]Model 314 E, Packard Instruments, LaGrange, Illinois.

high voltage setting of 5950 volts, and discrimator settings at 100-1000 on the red channel and 100-1000 on the green channel with a gain setting of 1000. A counting efficiency of 12.8% was observed on the green channel. The average marker progesterone recovery rate for the 20 pooled luteal homogenates was 28.54% ± 20%.

The mean progesterone content per gram of luteal tissue was calculated for each day of a 20 day estrous cycle. Progesterone levels for each two consecutive days, starting with day 1, of the cycle were combined and averaged, Table VI. The means and standard-deviations were calculated according to the basic methods outlined by Ostle (215).

CHAPTER IV

RESULTS

In order to fulfill the initial objectives set forth, a four part experiment was completed and the following determined. 1) Gross ovarian changes; 2) Luteal and endometrial phosphatase changes; 3) Pituitary and plasma gonadotropin concentrations; and 4) Endogenous luteal progesterone concentrations.

It was intended that these divisions of this experiment should provide a better understanding of the overall temporal relationships which exist between morphological changes, phosphatase enzyme activity changes and certain endocrine concentrations during the various stages of the estrous cycle, and thereby provide a contribution to the understanding of estrous cycle control mechanisms.

Part I

Gross Ovarian Changes: A knowledge of the gross ovarian morphological changes during a typical estrous cycle are essential in order to study the temporal relationships between the anterior pituitary gland, ovary and uterus in the normal porcine estrous cycle. Therefore, in this portion of the experiment, a sequence of photographs was made showing gross ovarian morphological changes in a predetermined 20-day estrous cycle.

Seventy-nine pairs of ovaries were photographed representing 3.95 gilts for each day of the estrous cycle. A series of 20 photographs

were selected which best illustrated the typical ovarian changes noted in all 79 pair. The series of ovarian pairs are shown in figure one.

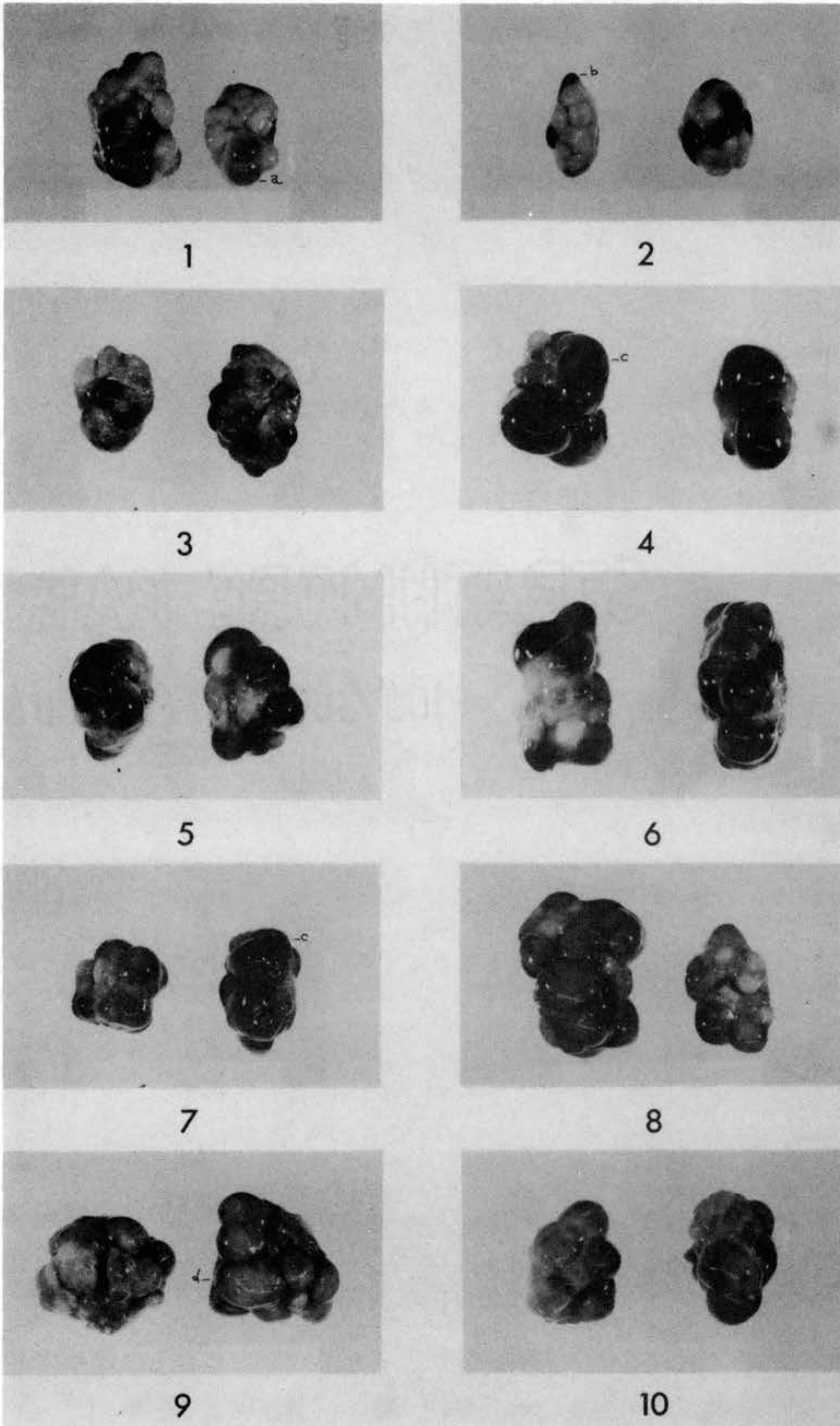
Forty-two per cent of all gilts slaughtered on days one, two and three of the estrous cycle, ovulated between days two and three. It was observed that none had ovulated on day one of the cycle. One out of four slaughtered on day two and all of those slaughtered on day three had ovulated. The pair of ovaries shown in figure 1 for day 2 demonstrate the morphological changes of recent ovulation.

A marked rapid growth of corpora lutea occurred between day 2 and day 4 followed by a change in luteal tissue from a soft "strawberry like" color and consistency to a firm "liver-like color and consistency. Between days 2 and 8 the corpora lutea appeared to mature, as indicated by the appearance of a superficial vascular network. The initial growth of many follicles 4 to 6 mm. in diameter was also observed.

Day 9 through day 14 marked a period in which few gross changes were noted. Corpora lutea attained maximum size and vascularity based on luteal weights and extensive vascularization while follicle size remained constant.

Between days 15 and 20 significant changes in the gross appearance of corpora lutea occurred. It was observed (figure 1) that ischemia of corpora lutea began on day 15 and became very pronounced by day 17. The color of corpora lutea changed from a "liver color" on day 14 to pale yellow or white by day 18. Also the size of degenerating corpora lutea decreased substantially by day 20. A portion of the large follicle crop noted on day 8 and maintained through day 14 began to enlarge significantly as the mass of luteal tissue decreased during the last stage of estrous cycle. Figure one illustrates the gross luteal and ovarian changes noted above.

FIGURE 1. (x 1.8) Photographs of 20 pairs of nulliparous gilt ovaries selected to illustrate typical gross ovarian changes each day of a 20-day estrous cycle. Key changes were noted by the following legends: a) mature follicle; b) recent ovulation; c) developing corpus luteum; d) mature corpus luteum; e) onset of luteal regression (paling); f) corpus albicans; g) mature follicle. The arabic numbers 1-20 represent respective days of the estrous cycle.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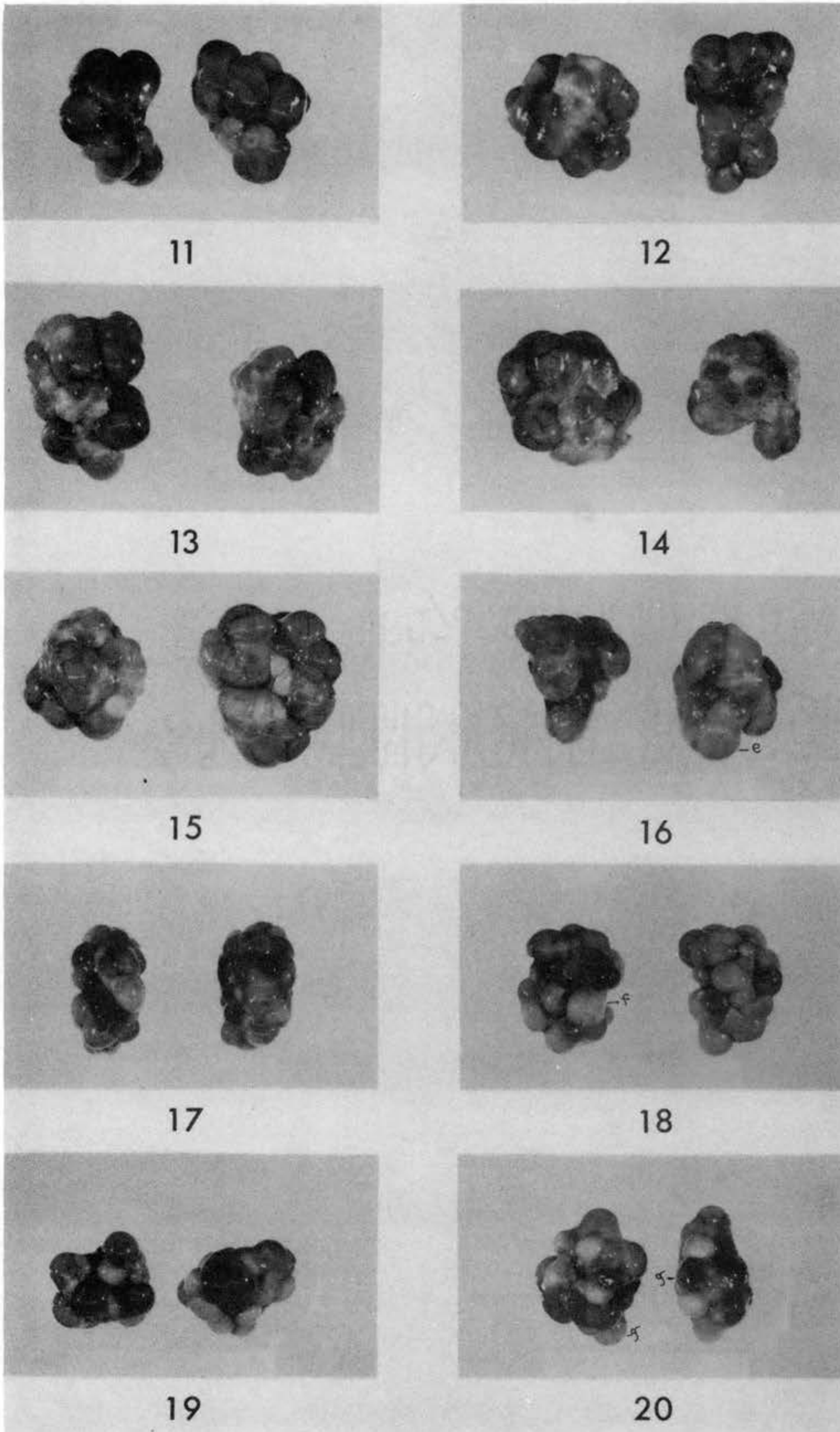
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Part II

Luteal and Endometrial Phosphatase Changes: To further study the temporal relationships between anterior pituitary, ovary and uterus during the normal porcine estrous cycle, the second part of this experiment was completed to compare daily changes in luteal and endometrial tissue phosphatase activities.

Table I

LUTEAL PHOSPHATASE ACTIVITY DURING VARIOUS STAGES OF THE ESTROUS CYCLE IN GILTS

Day of Cycle[1]	No. of Gilts[2]	Acid Phosphatase[3] Activity[4]	Alkaline phosphatase[3] Activity[4]	Ratio[5]
2-4	9	0.42 ± .03	0.46 ± .07	0.90
5-6	8	0.52 ± .05	0.43 ± .12	1.21
7-8	6	0.58 ± .08	0.36 ± .13	1.64
9-10	8	0.52 ± .06	0.29 ± .04	1.79
11-12	8	0.77 ± .13	0.53 ± .08	1.46
13-14	7	0.64 ± .06	0.66 ± .17	0.97
15-16	9	0.79 ± .12	0.68 ± .16	1.16
17-18	9	1.34 ± .39	0.41 ± .15	3.26
19-20	9	1.10 ± .19	0.16 ± .04	6.82
1-2	6	1.05 ± .17	0.19 ± .12	5.51

[1]In group 1-2, corpora albicans of the previous cycle were analyzed.

[2]Enzyme activities for each day were combined and averaged.

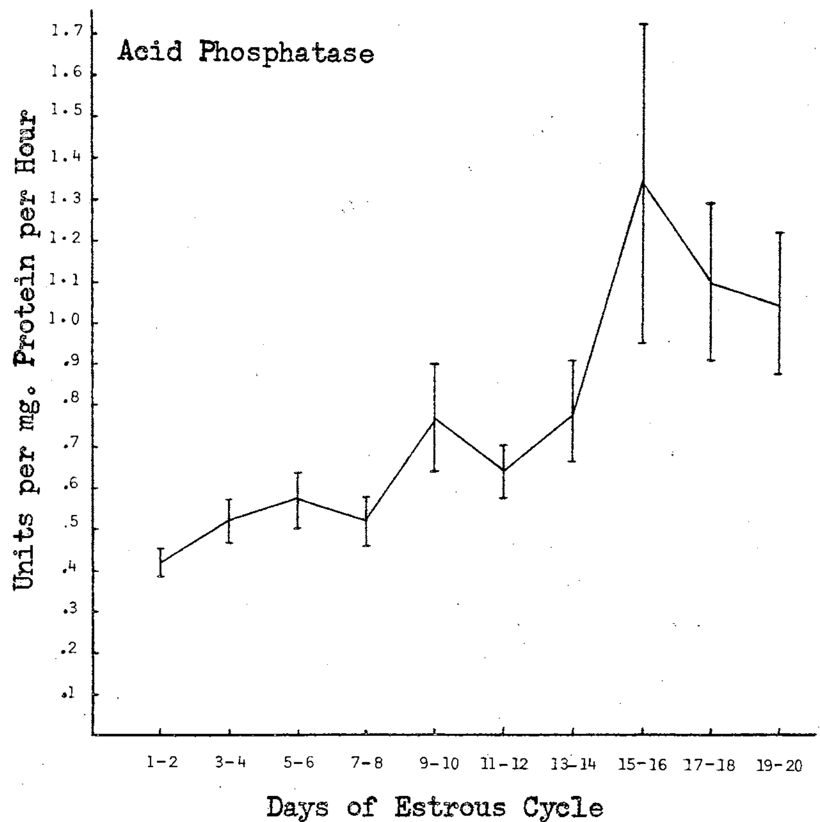
[3]Mean enzyme activity (± S.D.) expressed as Sigma units per mg. protein.

[4]Sigma unit is that amount of phosphatase which liberates 1 umole of p-nitrophenol per hour.

[5]Ratio of acid to alkaline Sigma units.

Chemical assay of alkaline and acid phosphatase activity changes in porcine corpora lutea revealed that acid phosphatase activity was greater than alkaline phosphatase activity throughout most of the estrous cycle. The results are shown in table I and Figure 2.

The acid and alkaline phosphatase activity levels were significantly



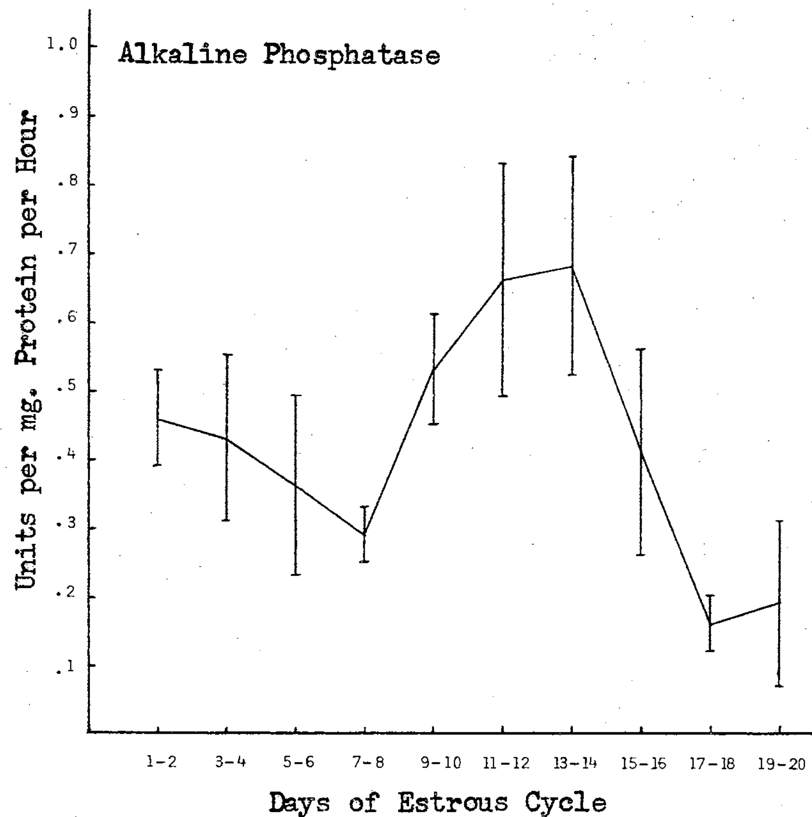


Figure 2. Acid and Alkaline Phosphatase Activity (Sigma Units) Trends in Luteal Tissue of Gilts During Consecutive Days of a 20-Day Estrous Cycle. Data Were Assembled in Groups of 2 Each and the Mean Activity Measured. Vertical Bars Represent the Standard Deviation of the Means.

Table II

ANALYSIS OF VARIANCE FOR LUTEAL ACID AND ALKALINE PHOSPHATASE ACTIVITIES DURING VARIOUS STAGES OF THE ESTROUS CYCLE IN GILTS

		Alkaline Phosphatase[1]			Acid Phosphatase[1]		
Source of Variation	DF	Sum of Squares	Mean Square	F-ratio	Sum of Squares	Mean Square	F-ratio
Total	79	23.6231			73.4286		
Mean	1	14.4792	14.4792		48.3495	48.3495	
Among (cycle days)	19	8.4316	.4438	36.6781**	27.5449	1.4497	34.6820**
Within (cycle days)	59	.7123	.0121		2.4658	.0418	

[1]Calculated from Sigma units per mg. protein for the respective enzymes of all animals concerned.

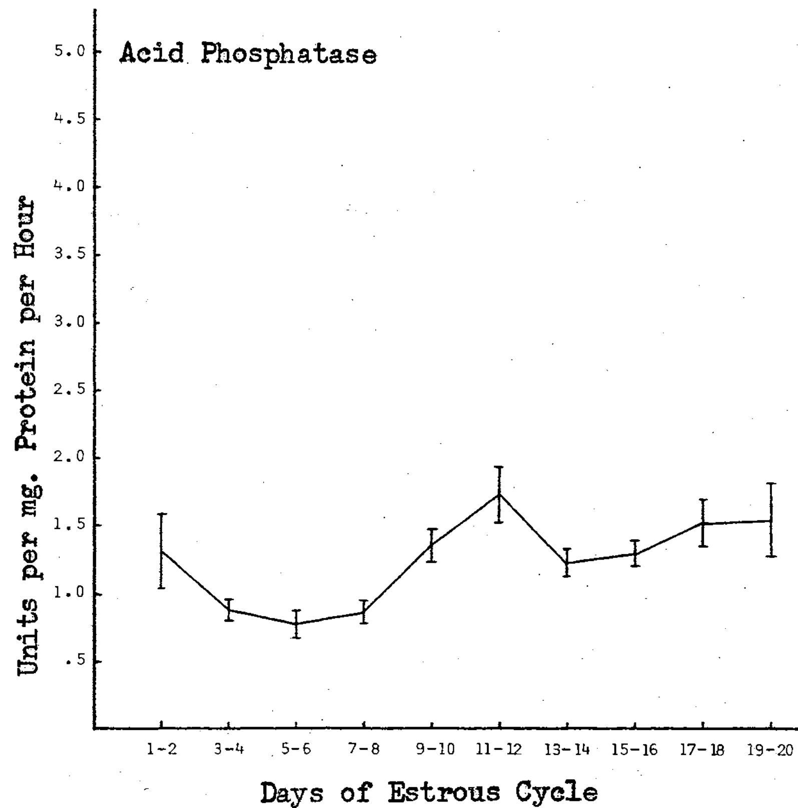
**($P < .01$)

different ($P < .01$) among estrous cycle days and not significantly different within cycle days (Table II). Acid phosphatase to alkaline phosphatase ratios were noticably greater than one on days 5-12 (1.21 to 1.79) and 17-20 (3.26 to 6.82) of the estrous cycle as shown in Table I. There was a progressive rise in acid phosphatase activity during day 6 (0.52 units) to day 12 (0.77 units) of the luteal phase. Whereas alkaline phosphatase activity steadily declined to day 10 (0.46 units to 0.29 units).

A marked change in the acid to alkaline phosphatase ratio occurred between days 14 and 17 of the estrous cycle. Gross observations, made in part 1 on these same corpora lutea, showed a definite ischemia at day 15 which became more pronounced as estrus approached.

Critical evaluation of luteal alkaline phosphatase activity (Table I) revealed an interesting trend in alkaline phosphatase as the estrous cycle progressed. Alkaline phosphatase activity progressively declined as corpora lutea matured, and reached a relatively low activity by day 10 (0.29 units), whereas a rapid rise in alkaline phosphatase activity occurred between days 10 and 15 of the cycle (Figure 2). There was a drastic decline in luteal alkaline phosphatase after day 15 (to 0.16 units).

Endometrial acid phosphatase activities changed little during the estrous cycle in gilts. However, figure 3 demonstrated that peak acid phosphatase activity occurred at day 12 which is the day that peak progesterone biosynthesis was found in this group of gilts and shown in part 4 of this experiment. The quantitative levels of acid phosphatase as measured by chemical assay throughout the cycle are recorded in Table III. Acid to phosphatase activity ratios were much greater than one during the endometrial regressive phase between days 17 (2.11) and 20



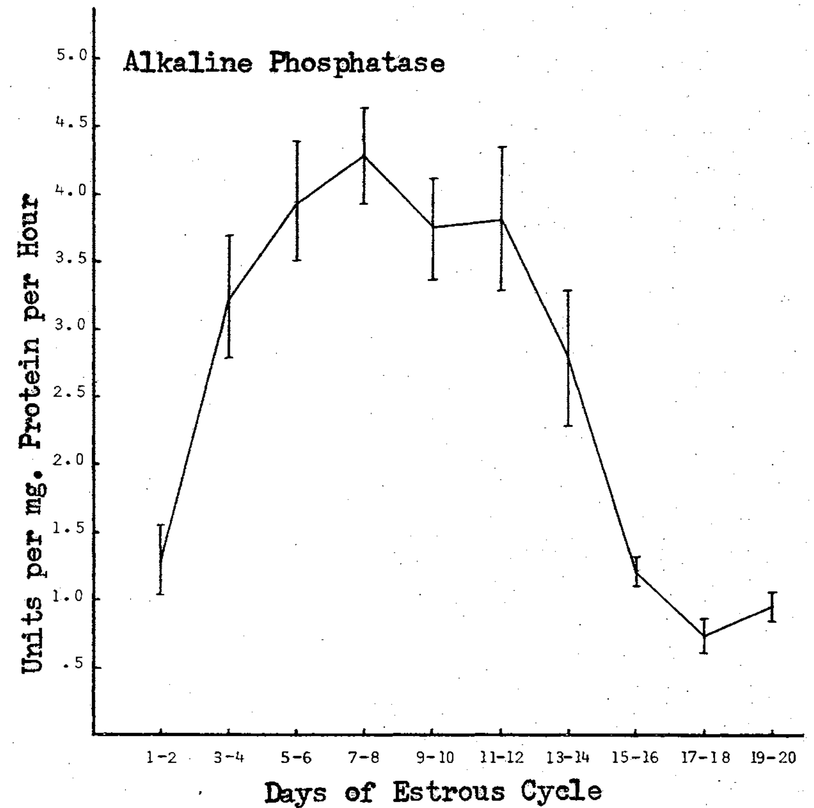


Figure 3. Acid and Alkaline Phosphatase Activity (Sigma Units) Trends in Endometrial Tissue of Gilts During Consecutive Days of a 20-Day Estrous Cycle. Data Were Assembled in Groups of 2 Days Each and the Mean Activity Measured. Vertical Bars Represent the Standard Deviation of the Means.

(1.64) of the cycle (Table III).

Changes in endometrial alkaline phosphatase activities during the estrous cycle were more dramatic. Figure 3 shows that high activity levels were quickly attained in the early stages of the estrous cycle. Peak alkaline phosphatase activity occurred on day 8, and a substantial decline in alkaline phosphatase activity followed between days 10 and 18 of the cycle. The respective phosphatase activity levels were significantly different ($P < .01$) among cycle days (Table IV).

Table III

ENDOMETRIAL PHOSPHATASE ACTIVITY DURING VARIOUS STAGES OF THE ESTROUS CYCLE IN GILTS

Day of Cycle	No. of Gilts[1]	Acid Phosphatase[2] Activity[3]	Alkaline Phosphatase[2] Activity[3]	Ratio[4]
1-2	7	1.32 ± .27	1.29 ± .26	1.03
3-4	8	0.88 ± .06	3.23 ± .46	0.27
5-6	8	0.77 ± .09	3.94 ± .46	0.20
7-8	6	0.86 ± .08	4.27 ± .37	0.20
9-10	8	1.36 ± .12	3.75 ± .36	0.36
11-12	8	1.75 ± .19	3.80 ± .53	0.46
13-14	7	1.23 ± .10	2.77 ± .50	0.44
15-16	9	1.30 ± .09	1.20 ± .10	1.08
17-18	9	1.53 ± .18	0.73 ± .12	2.11
19-20	10	1.55 ± .28	0.95 ± .10	1.64

[1]Enzyme activities for each day were combined and averaged.

[2]Mean enzyme activity (± S.D.) expressed as Sigma units per mg. protein.

[3]Sigma unit is that amount of phosphatase which liberates 1 umole of p-nitrophenol per hour.

[4]Ratio of acid to alkaline phosphatase activity (Sigma units).

Endometrial phosphatase changes appeared to precede luteal changes by 24-48 hours, if one compares the marked decline of alkaline phosphatase in the endometrium following day 12 (Table III) with the significant rise in acid phosphatase and the decline of alkaline phosphatase

Table IV

ANALYSIS OF VARIANCE ENDOMETRIAL ACID AND ALKALINE PHOSPHATASE ACTIVITIES DURING VARIOUS STAGES OF THE ESTROUS CYCLE IN GILTS

		Alkaline Phosphatase[1]			Acid Phosphatase[1]		
Source of Variation	DF	Sum of Squares	Mean Square	F-ratio	Sum of Squares	Mean Square	F-ratio
Total	80	702.8686			153.3998		
Mean	1	497.2388	497.2388		129.0498	129.0498	
Among (cycle days)	19	388.5153	20.4482	6.7085**	60.8632	3.2033	5.2634**
Within (cycle days)	60	182.8855	3.0481		36.5132	.6086	

[1]Calculated from Sigma units per mg. protein for the respective enzymes of all animals concerned.
**(P < .01)

activity following day 14 in luteal tissue (Table I).

Part III

<u>Pituitary and Plasma Gonadotropin Concentrations</u>: The third part of this experiment was designed to estimate FSH and LH concentrations in both plasma and pituitary tissue of gilts during each day of the estrous cycle.

Combined pituitary and plasma LH potency estimates are shown in Tables V and VI, and a graphic comparison of these estimates is illustrated in figure 4. It was observed that a marked rise in plasma LH occurred between days 1-2 and 5-6, while a relative drop in pituitary LH concentration took place during this period. Plasma LH reached a very high level at day 11-12 (6.69 ug/100 ml plasma) of the estrous cycle and then declined rapidly over the following 6 day period. Another sharp rise in plasma LH followed day 18 and continued until sometime before ovulation when it dropped to the low level noted on day 1-2 (2.30 ug./100 ml. plasma).

Pituitary LH concentration (Table V) increased progressively between days 3-4 (0.730 ug/mg dry pituitary) and 9-10 (2.057 ug/mg dry pituitary). Pituitary LH declined sharply by day 12 (1.220 ug./mg. dry pituitary) and then progressively increased as proestrus neared. The concentrations of plasma and pituitary LH tended to reach equal levels on day 18 (Figure 4). A marked rise in pituitary LH followed as the preovulatory phase developed, along with a substantial elevation in plasma LH.

Pituitary and plasma FSH potency estimates for each day of the estrous cycle for this group of gilts are shown in Tables V and VI. Figure 5 shows the comparison of mean plasma and pituitary potency

TABLE V

PITUITARY LH AND FSH CONCENTRATIONS DURING SUCCESSIVE PERIODS OF THE ESTROUS CYCLE IN GILTS[1]

Day of Estrous	No. of gilts	LH[2,3]		FSH[2,3]	
		Concentration	95% Limits	Concentration	95% Limits
1-2	6	1.34 ± .11	1.11 - 1.85	21.38 ± 3.79	13.02 - 29.65
3-4	8	0.73 ± .13	0.48 - 1.03	28.54 ± 3.94	20.67 - 37.16
5-6	7	1.31 ± .19	0.92 - 1.94	29.77 ± 3.96	21.73 - 38.72
7-8	5	1.75 ± .16	1.42 - 2.26	48.82 ± 5.07	39.81 - 61.04
9-10	8	2.06 ± .20	1.65 - 2.72	50.09 ± 5.16	40.94 - 62.62
11-12	6	1.22 ± .12	0.96 - 1.69	51.85 ± 5.87	41.22 - 67.34
13-14	7	1.33 ± .11	1.11 - 1.63	34.23 ± 4.99	23.71 - 48.33
15-16	8	1.78 ± .11	1.35 - 2.57	45.45 ± 5.21	37.20 - 63.48
17-18	8	2.32 ± .37	1.55 - 6.56	63.20 ± 6.32	56.46 - 89.32
19-20	9	3.42 ± .63	2.07 -14.24	39.97 ± 5.01	30.40 - 55.77

[1]Pituitary tissue was pooled within each cycle day.

[2]Each group represents the mean concentration (± S. D.) and 95% confidence intervals of all gilts in each 2 day period expressed as microgram-equivalents of NIH-FSH-S_1, or NIH-LH-S_1 per mg. of dry anterior pituitary.

[3]Combined precision estimate (g) for LH and FSH assays were respectively .146 and .136.

Table VI

PLASMA LH AND FSH CONCENTRATIONS DURING THE ESTROUS CYCLE IN GILTS[1]

Day of Estrous	No. of gilts	LH[2,3] Concentration	LH[2,3] 95% Limits	FSH[2,3] Concentration	FSH[2,3] 95% Limits
1-2	6	0.23 ± .07	0.08 - 0.35	43.70 ± 12.38	29.81 - 72.59
3-4	8	0.36 ± .01	0.15 - 0.68	38.50 ± 11.28	25.61 - 63.54
5-6	7	0.50 ± .12	0.26 - 0.88	49.65 ± 13.70	33.33 - 84.37
7-8	5	0.33 ± .08	0.16 - 0.55	42.05 ± 12.00	28.58 - 69.55
9-10	8	0.41 ± .09	0.22 - 0.71	46.30 ± 12.72	32.35 - 76.93
11-12	6	0.67 ± .14	0.39 - 1.36	51.60 ± 13.98	36.42 - 86.30
13-14	7	0.45 ± .10	0.25 - 0.83	53.65 ± 14.52	37.94 - 89.82
15-16	8	0.48 ± .11	0.26 - 0.86	52.37 ± 14.75	38.93 - 92.09
17-18	8	0.24 ± .07	0.10 - 0.39	60.95 ± 16.25	43.43 -102.52
19-20	9	0.45 ± .11	0.23 - 0.73	101.75 ± 26.04	72.89 -177.69

[1]Plasma was pooled within each cycle day.

[2]Each group represents the mean concentration (± S. D.) and 95% confidence intervals of all gilts in each 2 day period expressed as microgram-equivalents of NIH-FSH-S_1, or NIH-LH-S_1, per 10 ml. of whole plasma.

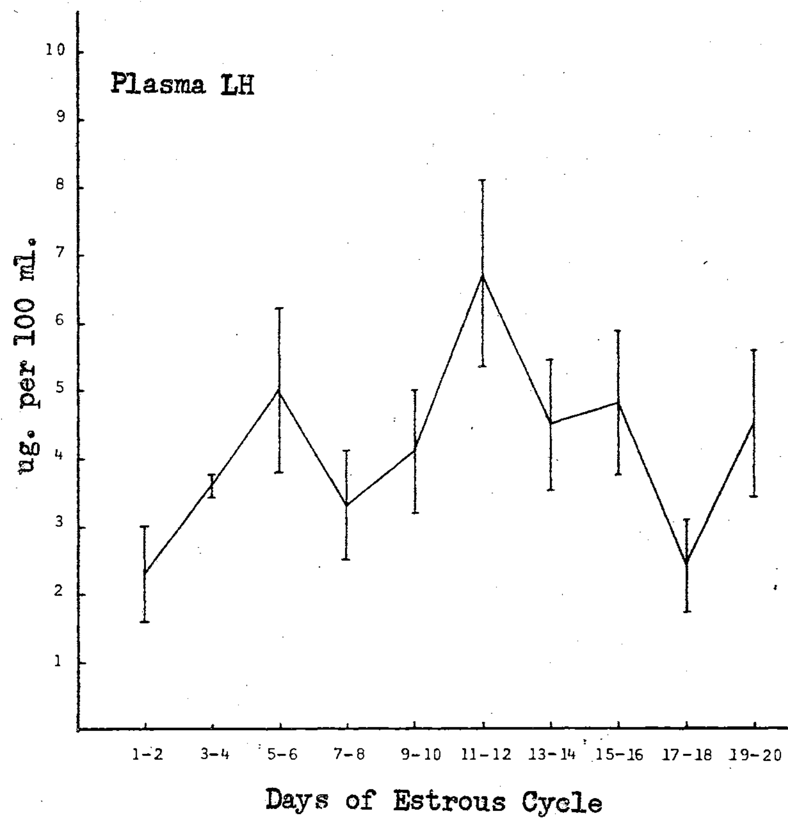
[3]Combined precision estimate (g) for LH and FSH assays were respectively .339 and .223.

during combined periods of the estrous cycle. Generally plasma FSH concentrations (ug/10 ml plasma) appeared to be very high in the porcine plasma (Table VI). The levels of plasma FSH remained rather constant between estrus (43.70 ug./10 ml. plasma) and days 17-18 (60.95 ug./10 ml. plasma) whereas pituitary FSH peaked at two distinct periods of the cycle (Figure 5). The first peak appeared at days 7-8 and lasted through days 11-12 and then declined, while the second peak occurred on days 17-18 and marked the highest concentration of pituitary FSH seen during the estrous cycle. A marked decrease in pituitary FSH followed day 18, while plasma FSH rose rapidly. These latter events appeared to be correlated with the pre-ovulatory follicle changes noted in part one.

The FSH to LH ratios for plasma are illustrated in figure 6. Plasma FSH concentrations tended to remain rather constant through day 18 of the cycle, while plasma LH fluctuated markedly at certain stages (Figures 4 and 5). The trend in plasma FSH to LH ratio was affected primarily by changes in LH concentrations.

Pituitary FSH to LH ratios shown in figure 6 demonstrated 3 prominent peaks during the estrous cycle. The peak reached on days 3-4 was due to a substantial drop in pituitary LH at that time (figure 4), and the peak ratio observed on days 11-12 was the result of a drop in pituitary LH coupled with a high level of pituitary FSH (Figure 4). The third peak seen on days 17-18 appeared to be due to the relatively rapid rise in pituitary FSH. After day 18 pituitary FSH concentrations declined rather rapidly and pituitary LH levels continued to rise sharply. This potency change in both FSH and LH accounts for the steep decline in the FSH-LH ratio seen on days 19-20.

One striking feature of the FSH-LH ratio was the marked change noted during the proestrus and estrus periods in plasma. These changes



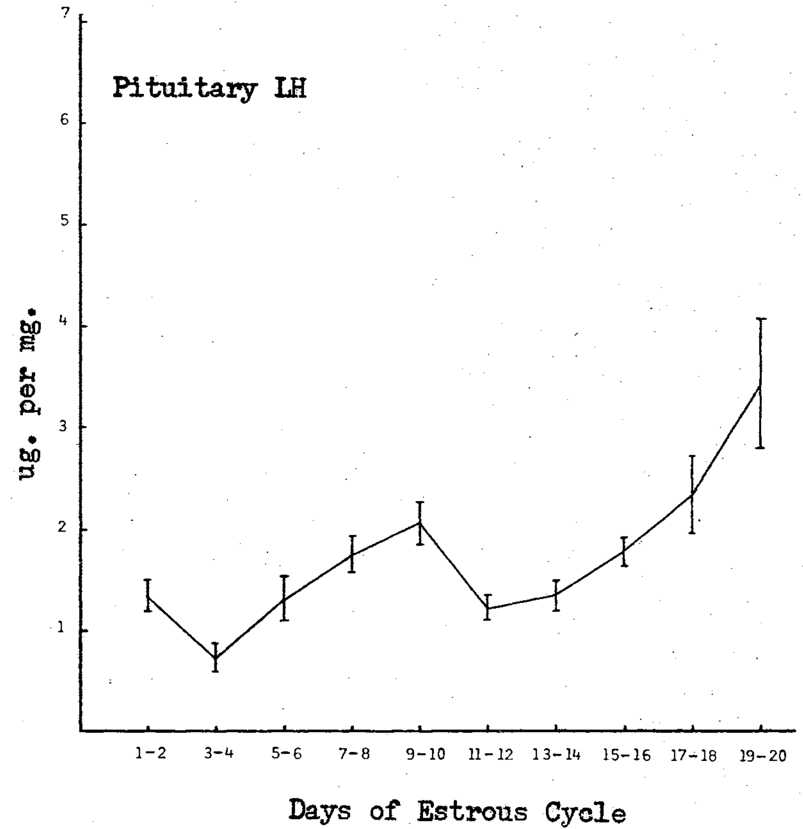
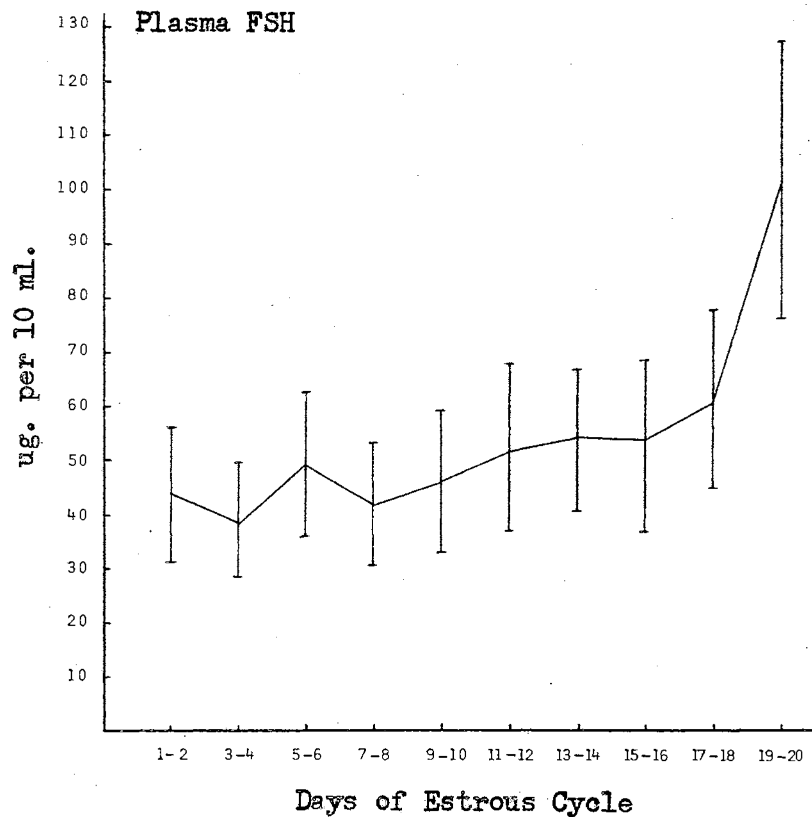


Figure 4. Blood Plasma and Dry Pituitary LH Concentration Trends During the Estrous Cycle in Gilts. Vertical Bars Represent the Approximate Standard Deviation of the Mean Relative Potency.



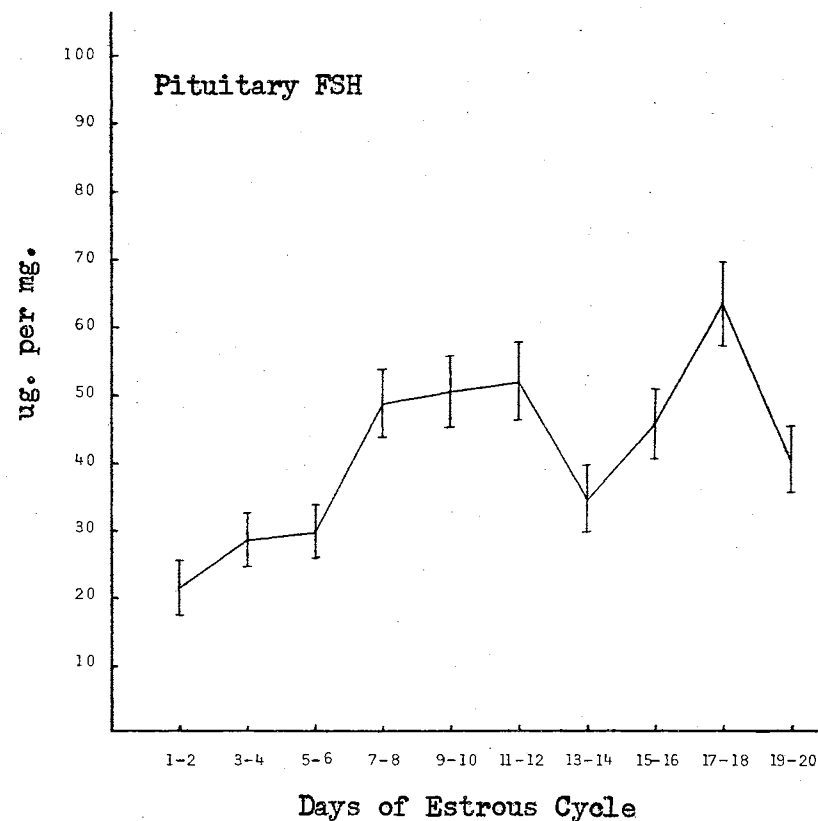


Figure 5. Blood Plasma and Dry Pituitary FSH Concentration Trends During the Estrous Cycle in Gilts. Vertical Bars Represent the Standard Deviation of the Mean Relative Potency.

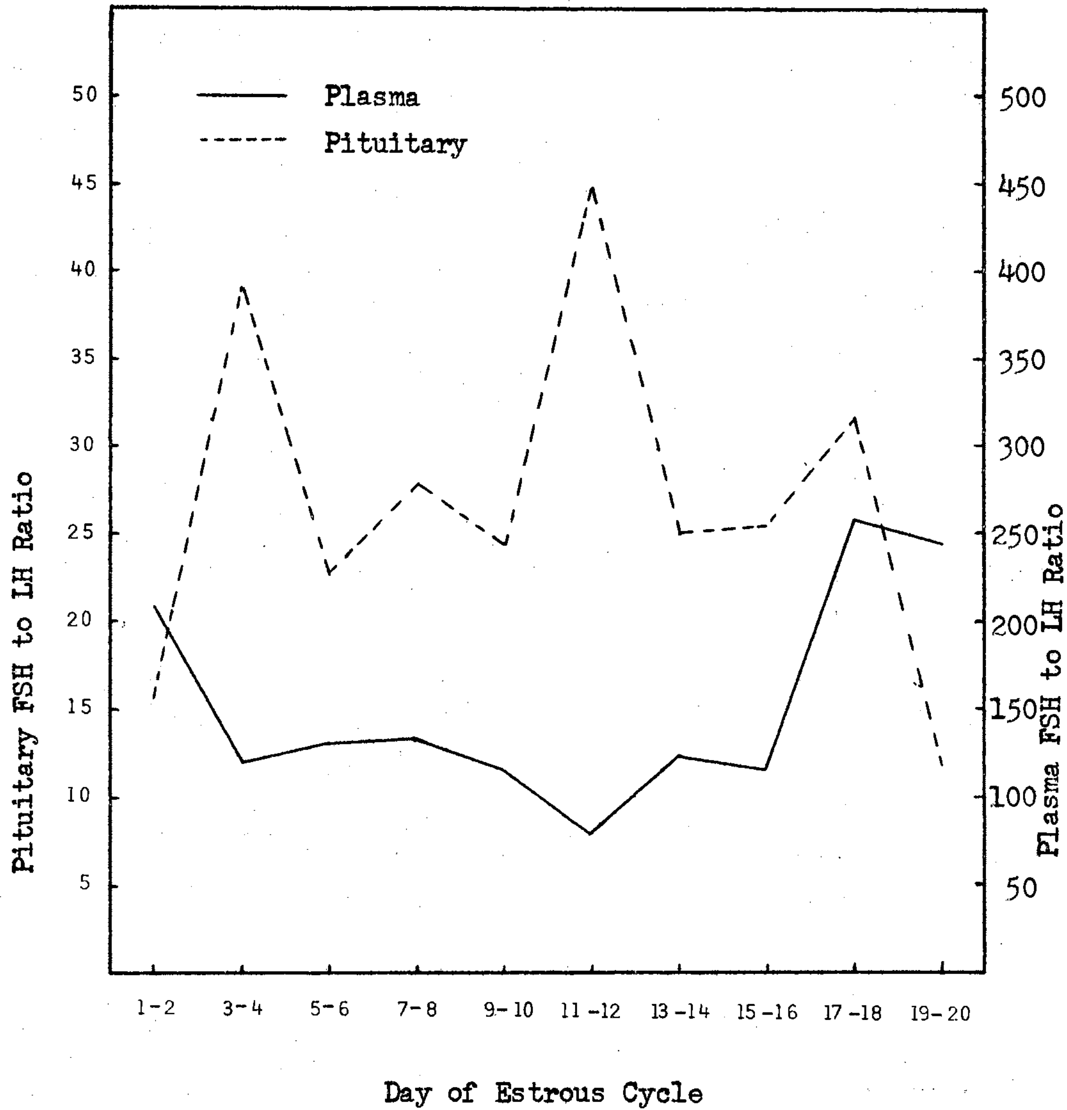


Figure 6. FSH-LH Ratio Trends in Blood Plasma and Dry Pituitary Tissue During the Estrous Cycle in Gilts.

reflected the rise in plasma FSH during proestrus and the sharp rise in LH during early estrus.

Another striking feature of the FSH-LH ratio in pituitary tissue was the separate peaks noted in figure 6 which appeared during each phase (metestrus, diestrus, and proestrus) of the estrous cycle. These ratio changes reflected in part, at least some of the morphological changes noted in experiment I, and some of the ovarian steriodogenic changes observed in the following part of this experiment.

Tables XII, XIII and XVI of appendix B contain the analysis of variance data on which the validity of each bioassay in this experiment was based. All HCG augmentation assays were valid as judged by the lack of significant variation caused by the blank treatment control rats and the zero intercept (Table XVI). The highly significant regression ($P < .01$) for all three HCG augmentation bioassays indicated that a large mean square for regression existed and therefore the relative precision (g) was very good (Tables V and VI).

The analysis of variance data for the OAAD bioassays in Tables XII and XIII indicated that all assays were valid as judged by the lack of significant deviation from parallelism. However bioassay 12A of Table XIII showed a nonsignificant regression. This indicated that the standard and test dose response linear regression lines were parallel and valid, but had very little slope. Therefore the potency estimate of plasma LH used in this experiment for day 6 was taken from bioassay 13A. Day 4 potency calculations were based on bioassay 12A.

Part IV

<u>Luteal Progesterone Concentrations</u>: The fourth part of this experiment was designed to measure the total luteal progesterone content in

pooled samples for each day of the estrous cycle. The corpora lutea from seventy-three gilts assigned to a 20 day cycle. Twenty pooled samples were extracted and then assayed for progesterone.

The mean corpora lutea weight from both ovaries illustrated the rate of luteal growth and regression during the estrous cycle in the pig. Corpora lutea attained their greatest mean weight on days 13-14; then declined sharply after days 15-16 (Table VII). At the 17-18th days the mean corpora lutea weight was half the peak value of days 13-14, which illustrated the abrupt degenerative changes noted in part one.

Table VII

CORPORA LUTEA PROGESTERONE CONCENTRATION DURING THE ESTROUS CYCLE IN GILTS[1]

Day of Cycle	No. of Gilts	Progesterone Concentration[2]	Corpora Lutea Weight[3]
1-2	6	trace	0.75 ± .17
3-4	8	38.65 ± 0.75	1.94 ± .42
5-6	7	32.45 ± 5.84	3.14 ± .36
7-8	6	36.90 ± 5.60	4.15 ± .40
9-10	8	35.30 ±14.49	4.76 ± .51
11-12	6	60.20 ±16.49	5.68 ± .43
13-14	7	40.35 ±13.10	6.14 ± .28
15-16	8	46.45 ±11.00	4.87 ± .60
17-18	8	12.13 ± 2.54	3.48 ± .45
19-20	9	0.69 ± 0.17	2.19 ± .39

[1]Luteal tissue was pooled for each cycle day and individual day progesterone concentrations were combined and averaged for each 2 consecutive days.

[2]Mean concentration (± S. D.) expressed as micrograms per gram wet tissue.

[3]Mean weight (± S. D.) expressed in grams.

The pattern of luteal progesterone concentration observed during the estrous cycle is shown in Table VII and further demonstrated in figure 6. Each point on the curve in figure 6 represents the combined

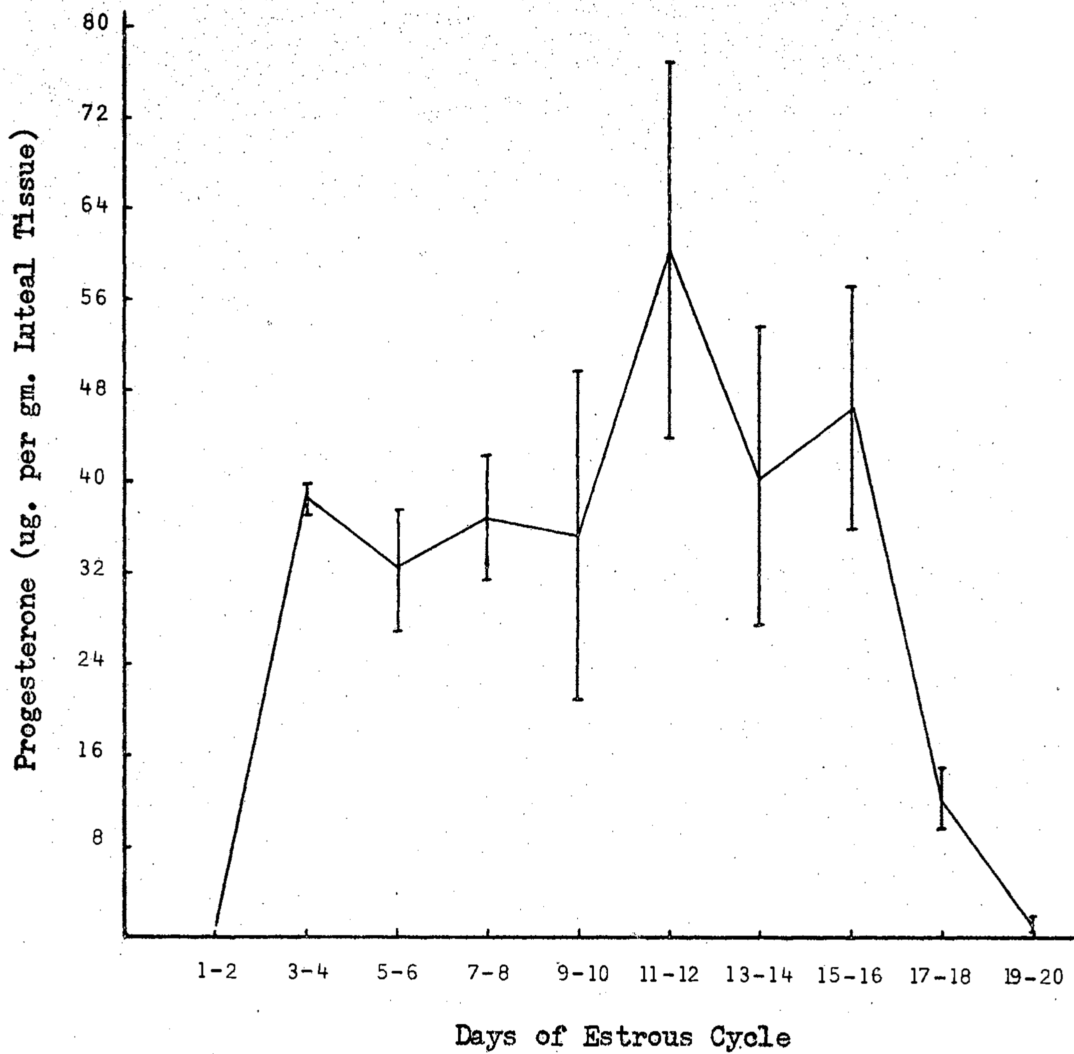


Figure 7. Luteal Progesterone Concentration Trends During the Estrous Cycle in Gilts. Vertical Bars Represent the Standard Deviations of the Mean.

mean quantity of progesterone in micrograms per gram of wet luteal tissue. The mean luteal progesterone concentration of 38.65 mcg/gm of tissue was rapidly attained by days 3-4, and this concentration was maintained through days 9-10 Table VII). A marked rise in luteal progesterone occurred at days 11-12 (60.2 ug./gm.). This peak was then followed by a rapid decline after days 15-16 which closely paralleled the plasma LH trends noted in part three.

CHAPTER V

DISCUSSION

Part I

Ovarian Changes During the Estrous Cycle: The series of photographs shown in Figure 1 depict ovarian changes for each day of a 20-day estrous cycle.

Critical examination of Figure 1 suggested three major morphological changes during the ovarian cycle. The first change started at ovulation (day 2) and extended through day 8. During this period of time the corpora lutea grew rapidly and attained maturity, while corpora albicans from the previous cycle continued to regress. Also it was observed that initial growth of many follicles started by day 3 and attained a certain static size on about day 8.

Since gilts were observed for estrus only once daily and slaughtered on their assigned day at 1 PM, many of the hourly changes observed by Hunter (20) and recorded in color photographs were not observed in this experiment. However ovaries collected on day 2 were almost identical in appearance to the recently ovulated porcine ovaries shown by Hunter (20).

The second change started on day 9 and extended through day 14 of the estrous cycle. During this period of the cycle, corpora lutea attained maximum mass, and peak progesterone concentrations which agreed with those reported by Masuda (124). Corpora lutea in this experiment

were typically "liver colored" and very vascular suggesting highly active tissue. Gross morphological changes were somewhat discrete during this period. Corpora albicans from the previous cycle became progressively smaller as the 40-day average life span was approached (24). The actual dimensions of corpora lutea were not taken in this experiment, although Part IV provided evidence that these corpora lutea attained their maximum weight between days 12 and 14 (Table VI).

The third change was that stage of the estrous cycle in which luteal regression predominated. The slightly ischemic appearance of day 15 ovaries noted in Figure 1 agreed with Corner's (24) observations in the sow ovary. The ovarian color changes described by Burger (16) clearly fit the changes observed in this experiment between days 15 and 18. These luteal degenerative changes were directly related to the decline in progesterone concentrations (Part IV) seen in both plasma and luteal tissue during this luteal regression phase.

Follicular growth suddenly resumed after day 16 indicating a response to the increasing amounts of plasma FSH which became available to the partially developed follicles (Table V). Change three included late diestrus and proestrus, and was a period in which ovarian morphology showed marked changes in appearance as did the uterus. This change was also related to observed changes in behavior.

The classical divisions of the estrous cycle (estrus, metestrus, diestrus and proestrus) can be related to the morphological changes described herein and which agree with other reports (17, 18). In Figure I, days 1 and 2 correspond to estrus. Days 2 through 4 typify the metestrus period. Days 5 through 15 show the peak luteal phase or diestrus period. While days 16 through 20 depict ovarian changes during proestrus. Day 2 overlaps estrus and metestrus since gilts and sows

generally ovulate while showing physical signs of estrus (16).

Part II

Luteal and Endometrial Phosphatase Changes: Acid and alkaline phosphatase activities per milligram of protein per hour were determined in luteal homogenates for each day of the porcine estrous cycle (Table I). Acid phosphatase activity was significantly higher than alkaline phosphatase between days 5 and 20 of the estrous cycle (Figure 2). There was a progressive rise in acid phosphatase activity during days 6 to 12 of the diestrus period. This phase included the early luteal phase in which coprora lutea were maturing and progesterone levels in the plasma and luteal tissue were rising (Table VII). Therefore, it was postulated that increased progesterone synthesis and secretion were related to the rising trend in acid phosphatase activity (221). The marked rise in acid phosphatase activity seen after day 14 reflected those luteal tissue degeneration changes which occurred at this time. Initiation of autolytic processes in corpora lutea may be due to some extra-ovarian agent or mechanism not yet identified which disrupts the local concentration of the lysosomal hydrolytic enzymes (221).

The marked shift in luteal acid to alkaline phosphatase ratios (Table I) observed after day 16 suggest some alteration in the metabolic activity of this tissue. This was reflected in the high acid phosphatase activity and the declining alkaline phosphatase activity (Figure 2).

The high levels of luteal acid phosphatase activity measured in this experiment prior to day 13 do not agree with the qualitative trends reported by others for the rat (150), cow (151), gilt (149) and woman (153). However, most of these results were based on histochemical analysis and not on chemical assay. Furthermore, in this experiment both

non-lysosomal and lysosomal acid phosphatases were measured (221).

Alkaline phosphatase activity in corpora lutea reflected luteal growth and function inversely prior to day 10, and directly after day 10 (Figure 2). Quantitatively, alkaline phosphatase activity was related to the period of high luteal progesterone concentration (Table VII) and showed a closer association with the classical phases of the estrous cycle than did luteal acid phosphatase. The early surge of alkaline phosphatase activity during the metestrus phase may be due primarily to the early infiltration of theca interna lutein cells during the differentiation of luteal tissue. Corner (147) demonstrated histochemically that theca lutein cells were the main source of alkaline phosphatase in porcine corpora lutea. It has also been shown that the theca interna cells make up a very small part of the mature luteal cell population in the non-pregnant sow ovary (24). Thus the gradual decline in alkaline phosphatase (Figure 2) through day 10 may be due to the increasing dominant function of the granulosa lutein cells. The surge in alkaline phosphatase activity observed between days 10 and 16 was postulated to be due to a shift in alkaline phosphatase production, and/or an increase in the theca lutein cell population. The rapid decline after day 16 reflected luteal cell degeneration (152).

Luteal acid phosphatase activity measured during the porcine estrous cycle reflected maturation and secretory activity of luteal cells during diestrus, and the rapid rise in late diestrus and proestrus indicated luteal regression. Whereas luteal alkaline phosphatase activity declined in metestrus and early diestrus, and reflected granulosa lutein cell maturation.

Endometrial acid phosphatase changed little during the estrous cycle (Table III). A rise in acid phosphatase activity occurred between

days 8 and 14, which was paralleled by a rapid decline in alkaline phosphatase. Talmage et al. (202) and Giering et al. (203) demonstrated that certain uterine metabolic activities were regulated by the ovarian hormones. Therefore, in this experiment it was postulated that changes in endometrial phosphatase levels reflected changes in uterine metabolism and that these changes were indirectly related to ovarian function. It has been reported in primates that rising estrogen levels potentiated synthesis of acid phosphatase in all areas of endometrial tissue and progesterone reversed this trend (204). But when the porcine uterus was under the influence of high progesterone levels, acid phosphatase predominated (209). Rising luteal progesterone levels (Table VII) and an assumed decline of estrogen influence until day 8 appeared to depress acid phosphatase levels (Figure 3). However, a new crop of ovarian follicles appeared by day 8, (Figure 1) and the upward surge of endometrial acid phosphatase between days 8-12 may have reflected the secretion of estrogen by these follicles.

The levels of endometrial acid and alkaline phosphatase activity in this experiment did not follow the qualitative proportions reported by Goode et al. (148,209). However the quantitative trend of acid and alkaline phosphatase demonstrated in Figure 3 was similar to those reported, which suggested that recovery rate of acid phosphatase may have been low for this tissue. Peak alkaline phosphatase activity occurred at day 8 perhaps reflecting an estrogenic influence of the preceding estrus period. Mansour et al. (207) demonstrated that gonadal estrogen acting on the endometrium can induce the surge of alkaline phosphatase noted here. A substantial decline in alkaline phosphatase took place after the maturation of luteal tissue between days 9 and 18 of the estrous cycle. The probable secretion of estrogen by the new crop of

follicles at day 8 may account for the plateau on the declining slope of the endometrial alkaline phosphatase curve (Figure 3). The sudden rise in luteal progesterone at day 12 reversed this influence again.

Corner (24) and McKenzie (18) described the cyclic histological pattern of the porcine endemetrial cells reverting from a tall columnar morphology at peak luteal function (day 12) to a low cuboidal morphology at late diestrus and early proestrus. Endometrial regression appeared to start sometime after day 16 in this experiment. This conclusion was based on the rapid decline of luteal progesterone noted in Figure 7, as well as the gradual rise in endometrial acid phosphatase.

In this experiment biochemical evaluation of phosphatase activities in the endometrium reflected gonadal hormone influences and indicated those periods when critical metabolic changes may have occurred. Whereas changes in phosphatase enzyme activities in corpora lutea indicated luteal development and the onset of luteal regression. Endometrial phosphatase changes appeared to precede luteal phosphatase changes by 48-72 hours. This was demonstrated by the marked decline of endometrial alkaline phosphatase following day 12, and the marked variation of acid and alkaline phosphatase activity in luteal tissue, respectively, following day 15.

Part III

Pituitary and Plasma Gonadotropin Concentrations: Quantitation of LH and FSH in blood plasma and pituitary tissue has become a necessary step in evaluating the regulatory mechanisms which exist between the ovary and anterior pituitary during the estrous cycle. Investigators are concerned with potencies, FSH-LH ratios and the time of FSH and LH release as related to ovulation and luteal development, function and

regression.

The type of biological assays used in this experiment were the indirect assays in which a quantitative response was measured. The ovarian ascorbic acid depletion (OAAD) response to LH has a log-dose response relationship and therefore the parallel-line assay analysis was the design of choice for potency calculation and validity evaluation (217). In validity analysis of the OAAD assay the condition of similarity required that the linear regression lines for the standard and test preparations be parallel within statistical limitations. Whereas in the HCG-augmentation FSH assay an arithmetic-dose response relationship existed and the slope-ratio analysis design has been found better suited for potency and validity evaluation of this bioassay. The slope-ratio linear regression lines for standard and test preparations should intersect at zero dose within statistical limits in order to be valid assays (217).

Pituitary LH potency estimates in this experiment agreed very well with those reported by Parlow *et al*. (96) for selected periods of the estrous cycle. However pituitary FSH concentrations were somewhat higher than those reported, although the trends were similar (96). Figure 4 shows that pituitary LH reached its lowest concentration on days 3-4. Release of LH was apparently augmented, as noted by the rising plasma level of LH (Figure 4). Turner (79) indicated that rising estrogen blood levels suppressed release of FSH and facilitated release of LH. It is also possible that the high blood levels of LH near ovulation may have fed back on the pituitary and hypothalamus and thereby assisted in the decreased synthesis of this hormone thus decreasing pituitary LH further (122). The decreasing plasma levels of FSH and rising pituitary FSH levels observed in this experiment correlated with these proposals

(Figure 5).

Figures 4 and 5 demonstrated that following days 5 and 6 both the pituitary LH and FSH mean relative potencies rose to significantly higher levels while plasma FSH remained rather constant and plasma LH mean relative potencies were erratic. This same relationship between days 4 and 10 was reported by Parlow (96) for the porcine estrous cycle. This corresponded to the development of a new crop of follicles as depicted in figure 1 and in figure 7 in progesterone concentrations. Rothchild (81) proposed that an increase in pituitary FSH-LH potency was induced by the rising blood progesterone levels between days 6 and 10. He did not postulate whether this increase was due to inhibition of release or increased synthesis.

The observation was made that plasma LH levels rose very high by day 11 of the estrous cycle and pituitary LH concentrations declined (Tables V and VI). This suggested that release of LH was induced just before these trends were noted. Neill _et al._ (121) observed that rapidly rising plasma progesterone levels initiated a rise in plasma LH concentrations. The rising progesterone concentrations seen at midcycle in this group of gilts (Table VII) may have triggered the release of LH from the pituitary and the peak progesterone concentrations recorded on day 12 may have occurred regardless of this release. The significance of high plasma LH levels at this stage of the estrous cycle and the effect on luteal function was not understood. Nevertheless, this period represented a critical time in the estrous cycle and additional FSH-LH measurements in pregnant gilts may indicate the significance of this positive relationship between plasma LH and progesterone concentrations (201).

A significant decline in FSH to LH ratio (figure 6) took place in

plasma and pituitary tissue on days 11-12 of the cycle. This reflected the marked change in LH at that time. Pituitary LH declined markedly causing the high pituitary ratio while plasma LH increased and resulted in the low plasma ratio. Again on days 15-16 a marked rise in the plasma ratio occurred (Figures 4 and 5) indicating key changes in FSH and LH concentrations after day 18. Blood levels of progesterone declined rapidly after day 16, whereas plasma LH rose again after day 18 (124). This suggested a negative relationship between progesterone and LH levels. However it was assumed that estrogen blood levels were rising rather rapidly at this time and that the estrogen-progesterone ratio complicated the mechanism operating during proestrus (119). Pituitary and plasma FSH potencies reflected the classical postulations reported during the proestrus period (79). Plasma FSH rose to very high levels while pituitary FSH declined.

The relative potencies of plasma LH determined in this experiment were within the bounds of those reported by Anderson and McShan (118). However plasma FSH potencies seemed very high in this experiment as compared to values reported for the bovine (222). Igarashi _et al_. (100) found that serum FSH levels in women were very high during the follicular phase and averaged 42.63 ug. of NIH-FSH-S2 equivalent per 10 ml. serum. Similar levels (Table V) were detected in gilts in this experiment. The explanation for the high concentrations of plasma FSH recorded in this experiment may be in one or more of the following theories. First, large amounts of porcine plasma were injected into each assay rat, and thus the accompanying quantities of LH may have augmented the ovarian response. Second, the porcine plasma may have elicited a gonadotropin release from the rat pituitary and thus augmented the response. Third, the half-life of FSH may be longer in swine than in some of the other species and this

may in turn account for the high levels of FSH. Fourth, free and protein-bound forms of FSH were measured since freezing and thawing may have freed most of the bound FSH. And fifth, these high FSH levels may actually represent those present in porcine plasma. Further investigations are needed to verify these postulations.

Part IV

Luteal Progesterone Concentrations: The progesterone content of luteal tissue has been used by many investigators as a criterion of luteal function in the pig (85,123,124,159). A composite illustration of the daily luteal progesterone trend for the 79 gilts used in this experiment is shown in Figure 7. The trends and concentrations agree very well with those published by Gomes (123) and Masuda (124). Comparison of Figure 7 to Figure 4 revealed some close similarities between luteal progesterone and plasma LH especially for that period of the estrous cycle between days 9 and 18. The tissues analyzed and the results graphically represented in these two figures were from the same group of animals and, although analyzed by unrelated techniques, the concentration trends were nearly parallel. It appeared that a definite positive relationship existed between luteal progesterone and plasma LH levels through day 18 of the estrous cycle. However, during proestrus an inverse relationship appeared to exist.

The progesterone concentration per gram of luteal tissue rose very rapidly within 24-48 hours after ovulation (Table VII). This sudden rise corresponded to the very rapid growth noted on days 3 and 4 in Figure 1. Also Figure 4 showed that plasma LH rose and peaked on day 5-6. The rising plasma levels of progesterone coupled with the declining levels of estrogen may have promoted the release of LH noted at this

time. Following this rapid initial change in the progesterone-estrogen relationship there existed a period of predominating, but essentially unchanging progesterone influence. This period was indicated by a plateau of luteal progesterone concentrations between days 3 and 10 (Figure 7). Meanwhile, plasma LH decreased and pituitary FSH and LH levels constantly increased (Figures 4 and 5). At days 11-12 there was a significant drop in pituitary LH while plasma LH and luteal progesterone rose very high. The significance of the parallel rise in both plasma progesterone and plasma LH cannot be explained from this experiment. It was also observed in this experiment (Table XI) that plasma LH peaked on day 11 of the estrous cycle while luteal progesterone peaked on day 12 (Table XVIII). However the rapid increase in luteal progesterone on days 9 through 10 may have triggered the hypothalamic "cyclic" center and thus caused a release of pituitary LH (75).

The extremely rapid decline in luteal progesterone between days 15-16 and 17-18 paralleled the degenerative morphological changes (Figure 1) and the rapid increase in luteal acid phosphatase activity (Figure 2). Plasma LH declined during this period (Figure 4), while the plasma FSH-LH ratio increased sharply (Figure 6).

As the estrous cycle progressed plasma FSH remained relatively constant between days 1 and 18 (Figure 5). This period corresponded to peak luteal influence and suggested that a significant reduction of FSH release took place. However, it was postulated that by day 18 luteal regression had progressed to the point that progesterone no longer inhibited FSH release whereas follicular estrogen had increased and the estrogen-progesterone ratio then favored release of FSH. FSH formation as reflected by pituitary FSH content (Table V) suggested that declining levels of progesterone promoted peak FSH formation by day 18 (Figures 5

and 7).

It was apparent from this study that precise concurrent measurements of plasma gonadotropins, progestins, and estrogens are essential to the complete understanding of the complex relationships which exist between the pituitary, ovary and uterus.

CHAPTER VI

SUMMARY AND CONCLUSIONS

From a review of the literature it was concluded that evidence exists for a reciprocal relationship between ovarian growth and function, and hypothalamus-anterior pituitary function. Recently, much evidence has been presented in many species that a functional relationship of unknown character exists between the endometrium and corpora lutea as well. It has also become obvious that an integration of function between these primary sites is necessary for the initiation, maintenance and termination of each estrous cycle. The dominant influence from each site on estrous cycle control varies among species. The endometrium appears to control estrous cycle length in the porcine, whereas this influence is not obvious in primates. Therefore, it was felt that an experiment designed to measure and observe some of the temporal endocrine, enzyme and ovarian morphology changes which occur during a predetermined estrous cycle for a certain species would contribute to the knowledge of estrous cycle control mechanisms for species in general.

A four-part experiment was designed to determine the relationships between pituitary and plasma gonadotropin concentrations, luteal progesterone concentrations and luteal-endometrial phosphatase activity changes in a group of 79 nulliparous gilts for each day of a 20-day estrous cycle. An effort was made to relate these endocrine and enzyme changes to the onset of luteal regression.

Part one revealed that three major morphological phases occurred

during the ovarian cycle. Phase one began at ovulation, extended through day 8, and was characterized by luteal maturation and the growth of new follicles. Phase two started at day 9 and ended about day 14. During this period corpora lutea attained maximum mass, and peak progesterone biosynthesis, while follicle size remained constant. Phase three began with the ischemic appearance of corpora lutea on day 15 and ended at the onset of the following estrus. This period was characterized by luteal degeneration and pronounced follicular growth. The classical divisions of the estrous cycle (estrus, metestrus, diestrus, and proestrus) were related to these morphological phases.

Part two revealed that acid phosphatase activity in luteal tissue was generally higher than alkaline phosphatase activity throughout most of the estrous cycle. A progressive rise in acid phosphatase activity starting at day 6, reflected maturation and secretion processes within corpora lutea, whereas a sharp rise at day 14 suggested the onset of luteal degeneration.

Luteal alkaline phosphatase activity declined progressively as corpora lutea matured and was lowest on day 10. A significant increase in alkaline phosphatase activity occurred between days 10 and 15. The critical rise in alkaline phosphatase activity was related to peak progesterone synthesis by luteal tissue. Alkaline phosphatase activity diminished greatly after day 15 and coincided with luteal regression. A change in the acid and alkaline phosphatase activity ratio between days 13 and 16 appeared to indicate a critical period in the metabolic life of porcine corpora lutea.

Endometrial acid phosphatase activity was low when compared to alkaline phosphatase activity and changed little throughout the estrus cycle. Endometrial alkaline phosphatase activity increased markedly

during the active luteal phase and decreased below acid phosphatase activity during the follicular phase. The most pronounced drop in endometrial alkaline phosphatase activity occurred after day 10 and appeared to indicate decreased estrogen dominance.

Luteal regression started at days 13-14, while some endometrial changes occurred at days 11-12. This indicated a 24-72 hour lag between significant endometrial enzyme changes and luteal enzyme changes. Such a delay may be related to the induction of luteal regression. Acid and alkaline phosphatase enzyme activities were closely related to the cyclic hormonal influences which exist in the porcine ovary and endometrium.

In parts three and four of this study, anterior pituitary and plasma concentrations of FSH and LH, and luteal progesterone concentrations were measured for each day of the estrous cycle. The HCG augmentation assay method of Steelman and Pohley was used to measure FSH in both pituitary and lyophylized plasma while the OAAD assay method of Parlow was used to measure LH extracted from plasma.

During the estrous cycle, increases in plasma LH were usually accompanied by decreases in pituitary LH. Plasma and pituitary FSH concentrations did not appear to be related directly to luteal progesterone concentrations and both showed a gradual increase until day 18. On day 18 of the estrous cycle plasma FSH increased markedly while pituitary FSH decreased. This pattern was apparently related to preovulatory follicle development. Plasma FSH mean potencies appeared to be relatively high. The significance of this was not determined, but could explain the higher incidence of cystic follicles in swine when compared to other species. The FSH to LH ratios in the pituitary varied rather markedly with the major phases of the estrous cycle while the plasma FSH to LH

ratios were more constant except during the follicular phase of the cycle. Most fluctuations in FSH-LH ratios appeared to be the result of variation in LH concentrations.

Luteal progesterone concentrations increased quickly after ovulation, plateaued at day 4, increased again at day 10, peaked at day 12, and then declined sharply to low levels by day 20. Luteal progesterone and plasma LH concentrations followed the same pattern until day 18 of the estrous cycle.

It was concluded that gross luteal degenerative changes were directly related to, and followed critical changes in luteal phosphatase activities, whereas endometrial phosphatase enzyme changes appeared to precede luteal phosphatase enzyme changes and reflected ovarian activity. This sequence of events indicated that some mechanism, operating between the endometrium and corpora lutea "shut off" luteal function between days 10 and 12. This fact was later reflected in the endogenous luteal progesterone decline at day 14. Luteal progesterone concentrations and plasma LH concentrations were positively related during the active luteal phase. It appeared that progesterone controlled the release of LH and inhibition of FSH formation prior to the proestrus period. Whereas a critical estrogen-progesterone ratio appeared to be essential for the release of FSH and LH in the proestrus period.

It was also concluded that some substance of mechanism of the non-pregnant porcine uterus, or acting via the uterus, abruptly "shuts off" luteal function. The resulting hormone changes then initiate the various ovarian morphological changes, phosphatase enzyme changes, luteal progesterone changes, and gonadotropin changes observed in this experiment and that the hypothalamus, through an innate periodicity then initiates a new cycle.

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A P P E N D I C E S

Appendix A

Table VIII

LUTEAL ACID AND ALKALINE PHOSPHATASE ACTIVITIES FOR SUCCESSIVE DAYS OF THE ESTROUS CYCLE IN GILTS

		Alkaline Phosphatase		Acid Phosphatase		
Day of Cycle	Ear Tatoo	Specific Activity[1]	Total Activity[2]	Specific Activity[1]	Total Activity[2]	Total Protein[3]
1	241	0.11	19.89	1.23	223.93	182.50
	225	0.08	14.06	1.40	261.94	187.50
	1204	0.06	8.03	0.81	103.02	127.50
2	239	0.08	92.00	1.62	190.00	117.50
	90X	0.05	14.66	0.58	167.04	287.50
	278	0.06	21.35	0.65	226.10	350.00
	470	0.09	180.08	0.47	122.06	262.50
3	216	0.36	79.66	0.45	99.01	222.50
	60X	0.74	181.06	0.50	122.01	245.00
	1011	0.55	109.00	0.35	69.00	200.00
	40	0.63	103.03	0.51	83.04	162.50
4	292	0.20	34.00	0.43	72.93	170.00
	455	0.27	62.11	0.41	93.96	227.50
	1231	0.43	64.95	0.32	48.00	150.00
	213	0.52	89.53	0.39	66.93	172.50
5	850	0.33	75.99	0.48	108.29	227.50
	204	0.30	40.98	0.40	55.00	137.50
	75	0.25	66.88	0.37	98.98	267.50
	1031	0.46	83.22	0.50	91.98	182.50
6	822	0.35	26.03	0.71	53.03	75.00
	1076	1.24	198.08	0.57	91.04	160.00
	47	0.28	21.62	0.70	54.02	77.50
	1227	0.29	41.06	0.43	80.06	187.50
7	217	0.62	63.96	0.51	51.97	102.50
	97	0.23	22.04	0.92	89.99	97.50
	212	0.18	30.98	0.39	68.95	175.00
8	1230	0.59	64.79	0.68	74.36	110.00
	53	0.40	54.53	0.56	76.99	137.00
	488	0.13	20.48	0.44	70.08	160.00
9	89	0.11	23.09	0.57	116.03	202.50
	1235	0.40	55.00	0.52	72.05	137.50
	1085	0.33	49.05	0.40	60.00	150.00
	274	0.17	51.77	0.43	133.92	310.00
10	1233	0.32	35.52	0.36	39.96	111.00
	837	0.20	51.77	0.37	94.10	255.00
	1228	0.42	36.04	0.73	61.97	85.00
	1002	0.39	60.95	0.80	126.00	157.00
11	1078	0.37	59.96	0.54	88.08	162.50
	208	0.60	72.00	0.82	98.04	120.00
	290	0.25	91.88	0.45	169.88	375.00
	273	0.78	85.25	1.23	135.19	111.00
12	99	0.91	109.20	1.43	171.00	120.00
	40	0.44	112.71	0.73	187.17	255.00
	1019	0.46	105.11	0.51	117.99	230.00

Table VIII (continued)

Day of Cycle	Ear Tatoo	Alkaline Phosphatase Specific Activity[1]	Alkaline Phosphatase Total Activity[2]	Acid Phosphatase Specific Activity[1]	Acid Phosphatase Total Activity[2]	Total Protein[3]
	1047	0.42	161.98	0.46	117.87	387.50
13	51	0.61	125.98	0.81	167.25	207.50
	1084	0.29	60.90	0.63	132.09	210.00
	655	0.35	43.00	0.62	75.95	122.50
14	833	1.55	154.80	0.91	91.00	100.00
	1025	1.03	149.93	0.58	83.96	145.00
	813	0.51	85.93	0.65	108.04	167.50
	457	0.53	115.93	0.25	53.94	217.50
15	650	0.45	81.00	1.48	266.04	180.00
	96	0.35	90.56	0.67	175.88	262.50
	694	0.66	126.09	0.77	149.00	192.50
	46	0.59	110.06	0.66	123.94	187.50
	263	1.71	349.94	0.50	102.09	205.00
16	61	1.09	141.96	1.20	156.00	130.00
	1014	0.80	170.00	0.84	178.50	212.50
	875	0.34	96.89	0.33	96.03	287.50
	496	0.20	59.09	0.64	186.03	292.50
17	456	0.27	82.05	0.62	187.88	305.00
	270	0.24	101.05	0.92	396.03	430.00
	276	0.45	49.95	1.63	181.04	111.00
	452	0.81	180.00	0.40	89.89	222.50
18	472	0.05	19.55	0.49	207.83	425.00
	427	0.22	16.24	2.92	211.99	72.50
	271	1.42	39.00	3.78	104.01	27.50
	692	0.08	18.04	0.79	174.02	220.00
	45	0.12	23.25	0.91	170.06	187.50
19	218	0.06	13.28	0.75	156.04	207.50
	812	0.26	31.46	1.01	122.21	121.00
	607	0.32	28.18	1.98	173.25	87.50
	494	0.08	27.91	0.73	263.90	362.50
	864	0.15	17.51	1.24	146.05	117.50
20	815	0.09	33.82	0.89	337.82	380.00
	487	0.05	13.65	0.36	93.98	262.50
	855	0.30	30.96	2.09	214.02	102.50
	842	0.15	27.94	0.88	164.06	187.50

[1]Sigma units per mg. protein per hour.

[2]Sigma units per gm. wet tissue per hour.

[3]Milligrams per gm. wet tissue.

Table IX

ENDOMETRIAL ACID AND ALKALINE PHOSPHATASE ACTIVITIES FOR SUCCESSIVE DAYS OF THE ESTROUS CYCLE IN GILTS

		Alkaline Phosphatase		Acid Phosphatase		
Day of Cycle	Ear Tatoo	Specific Activity[1]	Total Activity[2]	Specific Activity[1]	Total Activity[2]	Total Protein[3]
1	241	1.24	97.97	1.30	102.38	78.75
	225	0.63	77.47	0.61	123.75	123.75
	1204	1.64	75.99	1.99	91.99	46.25
2	239	0.24	29.38	0.67	84.00	125.00
	90X	2.15	101.98	0.78	37.19	47.50
	278	1.28	80.00	2.51	157.00	62.50
	470	1.96	119.99	1.40	86.00	61.25
3	216	2.35	200.00	0.92	85.25	85.25
	60X	0.92	61.63	0.80	54.00	67.50
	1011	3.67	251.97	1.14	78.03	68.75
	40	4.55	290.00	0.85	54.00	63.75
4		2.23	220.02	0.69	68.04	98.75
	455	4.82	482.00	0.91	91.20	100.00
	1231	3.60	180.00	0.83	41.60	50.00
	213	3.72	329.97	0.90	79.96	88.75
5	850	4.50	270.00	1.07	64.02	60.00
	204	3.94	334.99	0.75	64.01	85.00
		2.58	335.01	0.22	28.34	130.00
	1031	5.24	484.98	0.95	87.97	92.50
6		2.44	192.47	0.76	59.25	78.75
	1076	3.50	205.63	0.96	56.40	58.75
	47	6.08	190.00	0.83	26.00	31.25
	1227	3.20	260.00	0.64	52.00	81.25
7	217	3.69	234.98	0.68	43.22	63.75
	97	4.18	470.03	0.62	69.98	112.50
	212	3.19	255.04	0.93	65.10	80.00
8	1230	5.68	525.03	0.95	87.97	92.50
	53	4.97	410.03	1.16	96.03	82.50
	488	3.93	294.98	0.82	62.03	75.00
9	89	4.30	322.50	1.31	98.03	75.00
		3.65	365.00	o.28	128.00	100.00
	1085	4.67	385.03	1.16	96.03	82.50
	274	3.83	344.97	1.09	98.01	90.00
10	1233	4.36	245.03	1.42	79.99	56.25
	837	3.25	247.51	1.14	87.00	76.25
	1228	4.35	375.02	2.13	183.97	86.25
		1.58	75.00	1.33	62.99	47.50
11		2.04	277.95	1.38	188.03	136.25
	208	4.14	212.02	2.22	113.98	51.25
	290	5.26	459.99	1.90	165.99	87.50
	273	3.61	370.03	1.33	136.02	102.50
12	99	2.68	157.51	1.77	103.99	58.75
	40	6.30	260.00	2.72	111.99	41.35
		2.24	260.05	1.12	129.97	116.25

Table IX (continued)

Day of Cycle	Ear Tatoo	Alkaline Phosphatase Specific Activity[1]	Alkaline Phosphatase Total Activity[2]	Acid Phosphatase Specific Activity[1]	Acid Phosphatase Total Activity[2]	Total Protein[3]
	1047	4.15	202.51	1.56	76.00	48.75
13	51	3.16	177.98	1.39	78.02	56.25
	1084	5.24	379.97	1.68	122.02	72.50
	655	1.70	182.97	1.17	125.99	107.50
14	833	2.08	197.22	1.18	112.00	95.00
		1.42	109.97	1.06	82.00	77.50
	813	2.03	190.03	0.88	82.03	93.75
	475	3.14	385.02	1.06	129.97	122.50
15	650	1.00	102.50	1.30	133.05	102.50
	96	1.54	96.00	1.38	86.00	62.50
	694	1.06	73.01	1.19	82.02	68.75
	46	1.38	149.97	0.92	100.96	108.75
	263	1.40	119.00	1.58	133.96	85.00
16	61	1.72	124.99	0.99	71.99	72.50
	1014	1.07	54.99	1.39	70.98	51.25
	875	1.02	62.48	1.75	107.00	61.25
	496	1.80	99.00	1.16	64.02	55.00
17	456	0.26	35.99	0.90	125.97	139.50
	270	0.75	36.01	1.94	92.01	47.50
	276	0.34	50.03	1.61	232.01	143.75
	452	0.78	74.98	2.22	213.96	96.25
18	472	1.46	74.62	1.37	70.01	51.25
	427	0.66	53.04	0.95	76.00	80.00
	271	0.84	59.01	1.80	126.00	70.00
	692	1.10	112.55	2.07	211.97	102.50
	45	0.54	65.04	0.80	96.00	120.00
19	218	0.77	79.03	2.07	211.97	102.50
	812	0.38	45.01	3.86	458.02	118.75
	607	1.42	79.99	1.03	57.99	56.25
	494	1.04	65.98	1.08	68.98	63.75
	864	1.20	75.00	1.60	100.00	62.50
20	815	0.70	70.00	0.98	98.00	100.00
	487	1.30	83.00	1.66	106.02	63.75
	855	0.69	42.02	1.11	67.99	61.25
	842	0.90	87.46	0.96	93.99	97.50
	487	1.01	96.03	1.12	100.98	90.00

[1]Sigma units per mg. protein per hour.

[2]Sigma units per gm. wet tissue per hour.

[3]Milligrams per gm. wet tissue.

Appendix B

Table X

PITUITARY LUTEINIZING HORMONE RELATIVE POTENCIES FOR SUCCESSIVE DAYS OF THE ESTROUS CYCLE IN GILTS[1]

Day of Cycle	Log 10 of Relative Potency (M)	Mean Relative Potency (R)[2]	95% Fiducial Limits
1	+0.935	1.471 ± .042	1.383 - 1.916
2	+0.366	1.207 ± .181	0.844 - 1.779
3	-1.172	0.709 ± .127	0.454 - 1.006
4	-1.005	0.751 ± .127	0.497 - 1.046
5	+1.004	1.506 ± .211	1.082 - 2.279
6	+0.103	1.103 ± .169	0.764 - 1.604
7	+1.262	1.648 ± .149	1.344 - 2.103
8	+1.611	1.860 ± .174	1.504 - 2.418
9	+1.734	1.940 ± .183	1.566 - 2.539
10	+2.062	2.173 ± .213	1.738 - 2.902
11	+0.027	1.074 ± .087	0.897 - 1.287
12	+0.723	1.367 ± .155	1.029 - 2.096
13	+0.298	1.180 ± .095	0.987 - 1.422
14	+0.964	1.486 ± .120	1.241 - 1.830
15	+1.326	1.685 ± .194	1.289 - 2.407
16	+1.619	1.865 ± .220	1.417 - 2.738
17	+1.243	1.627 ± .188	1.255 - 2.322
18	+2.990	2.998 ± .546	1.850 -10.787
19	+3.482	3.556 ± .694	2.098 -15.245
20	+3.249	3.280 ± .570	2.037 -13.239

[1]Results based on 8 ovarian ascorbic acid depletion bioassays and expressed as micrograms of NIH-LH-S_1 equivalent per mg. dry pituitary.

[2]Micrograms (± S. D.) of NIH-LH-S_1.

Table XI

PLASMA LUTEINIZING HORMONE RELATIVE POTENCIES FOR SUCCESSIVE DAYS OF THE ESTROUS CYCLE IN GILTS[1]

Day of Cycle	Log 10 of Relative Potency (M)	Mean Relative Potency (R)[2]	95% Fiducial Limits
1	-1.543	2.889 ± 0.793	1.274 - 4.090
2	-2.949	1.777 ± 0.669	0.414 - 3.084
3	+0.610	5.032 ± 1.022	2.970 - 9.501
4	-2.420	2.136 ± 1.032	0.005 - 4.184
5	-1.436	3.002 ± 0.891	1.188 - 4.880
6	+0.299	6.996 ± 1.458	4.056 -12.712
7	-0.495	4.138 ± 0.949	2.223 - 7.051
8	-2.109	2.383 ± 0.697	0.978 - 3.995
9	-0.998	3.472 ± 0.858	1.741 - 5.799
10	-0.082	4.728 ± 1.027	2.657 - 8.294
11	+1.515	8.064 ± 1.620	4.817 -17.502
12	+0.216	5.310 ± 1.135	3.035 - 9.635
13	-0.750	3.750 ± 0.909	1.959 - 7.038
14	+0.173	5.231 ± 1.123	2.980 - 9.458
15	-0.834	3.672 ± 0.894	1.880 - 6.220
16	+0.522	5.842 ± 1.218	3.402 -10.968
17	-1.977	2.470 ± 0.711	1.033 - 3.975
18	-2.190	2.307 ± 0.687	0.919 - 3.736
19	-1.492	3.689 ± 0.975	1.719 - 5.873
20	-0.437	5.284 ± 1.149	2.962 - 8.638

[1]Results based on 5 ovarian ascorbic acid depletion bioassays and expressed as micrograms of NIH-LH-S_1 equivalent per 100 ml. plasma.

[2]Micrograms (± S. D.) of NIH-LH-S_1.

Table XII

ANALYSIS OF VARIANCE FOR OVARIAN ASCORBIC ACID DEPLETION ASSAYS USED TO EVALUATE DRY ANTERIOR PITUITARY LH CONCENTRATIONS FOR SUCCESSIVE DAYS OF THE ESTROUS CYCLE IN GILTS

Source of Variation[1]	df	Sums of Squares	Mean Square	F Ratio
Total	19	11380.36		
Preparations	1	2175.70	2175.70	12.354**
Regression	1	6379.59	6379.59	36.222**
Parrallelism	1	7.20	7.20	0.041
Error	16	2817.87	176.12	

[1]Bioassay 01A (day 1, of pituitary)

**(P < .01)

Table XII (continued)

Source of Variation	df	Sums of Squares	Mean Square	F Ratio
Total	59	18330.30		
Preparations	5	4514.35	902.87	5.480**
Regression	1	5021.52	5021.52	30.476**
Parallelism	5	885.69	177.14	1.075
Error	48	7908.75	164.77	

[1]Bioassay 02A (days 2, 3, 4, 5, 6 of pituitary)

**(P < .01)

Table XII (continued)

Source of Variation[1]	df	Sum of Squares	Mean Square	F Ratio
Total	39	9509.09		
Preparations	4	3604.03	901.01	16.176**
Regression	1	3974.04	3974.04	71.347**
Parallelism	4	259.97	64.99	1.167
Error	30	1671.05	55.70	

[1]Bioassay 02B (days 7, 8, 9, 10 of pituitary)

**(P $<$ .01)

Table XII (continued)

Source of Variation[1]	df	Sum of Squares	Mean Square	F Ratio
Total	39	9744.19		
Preparations	3	1407.23	469.08	6.393**
Regression	1	5963.36	5963.36	81.278**
Parallelism	3	25.63	8.55	0.117
Error	32	2347.95	73.37	

[1]Bioassay 03A (days 11, 13, 14 of pituitary)

**(P $<$ .01)

Table XII (continued)

Source of Variation[1]	df	Sums of Squares	Mean Square	F Ratio
Total	15	1767.32		
Preparations	1	200.22	200.22	3.713*
Regression	1	861.42	861.42	15.973**
Parallelism	1	58.52	58.52	1.085
Error	12	647.15	53.93	

[1]Bioassay S3A (day 12 of pituitary)

**($P < .01$)

*($P < .01$)

Table XII (continued)

Source of Variation[1]	df	Sums of Squares	Mean Square	F Ratio
Total	39	13197.91		
Preparations	3	3178.02	1059.34	7.489**
Regression	1	5283.10	5283.10	37.350**
Parallelism	3	210.26	70.09	0.496
Error	32	4526.52	141.45	

[1]Bioassay 03B (days 15, 16, 17 of pituitary)

**($P < .01$)

Table XII (continued)

Source of Variation[1]	df	Sums of Squares	Mean Square	F Ratio
Total	23	2598.21		
Preparations	2	1599.39	799.69	25.077**
Regression	1	380.01	380.01	11.916**
Parallelism	2	44.84	22.42	0.703
Error	18	573.97	31.89	

[1]Bioassay 04A (days 18, 19 of pituitary)

**($P < .01$)

Table XII (continued)

Source of Variation[1]	df	Sums of Squares	Mean Square	F Ratio
Total	15	4664.76		
Preparations	1	3220.56	3220.56	57.654**
Regression	1	686.44	686.44	12.289**
Parallelism	1	87.42	87.42	1.565
Error	12	670.34	55.86	

[1]Bioassay 04B (day 20 of pituitary)

**($P < .01$)

Table XIII

ANALYSIS OF VARIANCE FOR OVARIAN ASCORBIC ACID DEPLETION ASSAYS USED TO EVALUATE PLASMA LH CONCENTRATIONS FOR SUCCESSIVE DAYS OF THE ESTROUS CYCLE IN GILTS

Source of Variation[1]	df	Sums of Squares	Mean Square	F Ratio
Total	39	10169.99		
Preparations	3	3020.04	1006.68	6.101**
Regression	1	1786.23	1786.23	10.826**
Parallelism	3	83.82	27.94	0.169
Error	32	5279.90	164.99	

[1]Bioassay 11A (days 1, 2, 5 of plasma)

**($P < .01$)

Table XIII (continued)

Source of Variation[1]	df	Sums of Squares	Mean Square	F Ratio
Total	29	12391.40		
Preparations	2	979.93	489.97	1.212
Regression	1	590.52	590.52	1.461
Parallelism	2	1120.46	560.23	1.386
Error	24	9700.49	404.19	

[1]Bioassay 12A (days 4, 6 of plasma)

Table XIII (continued)

Source of Variation[1]	df	Sums of Squares	Mean Squares	F Ratio
Total	55	30406.09		
Preparations	6	3116.29	519.38	1.033
Regression	1	3593.61	3593.61	7.148**
Parallelism	6	2580.52	430.09	9.855
Error	42	21115.68	.75	

[1]Bioassay 13A (days 3, 6, 7, 8, 9, 10 of plasma)

**(P < .01)

Table XIII (continued)

Source of Variation[1]	df	Sums of Squares	Mean Squares	F Ratio
Total	69	33539.71		
Preparations	6	4976.90	829.48	2.291*
Regression	1	5869.73	5869.73	16.221**
Parallelism	6	2428.76	404.79	1.119
Error	56	20264.32	361.86	

[1]Bioassay 14A (days 11, 12, 13, 14, 15, 16 of plasma)

**(P < .01)

*(P < .01)

Table XIII (continued)

Source of Variation[1]	df	Sums of Squares	Mean Squares	F Ratio
Total	49	16755.65		
Preparations	4	3835.17	958.79	4.195**
Regression	1	3166.49	3166.49	13.853**
Parallelism	4	611.08	152.77	0.668
Error	40	9142.91	228.57	

[1]Bioassay 15A (days 17, 18, 19, 20 of plasma)

**($P < .01$)

Table XIV

PITUITARY FOLLICLE STIMULATING HORMONE RELATIVE POTENCIES FOR SUCCESSIVE DAYS OF THE ESTROUS CYCLE IN GILTS

Day of Cycle	Slope Ratio (R)[1]	Mean Relative Potency[2]	95% Fiducial Limits
1	0.73 ± .10	28.81 ± 3.91	20.95 - 37.47
2	0.35 ± .09	13.95 ± 3.67	5.09 - 21.82
3	0.71 ± .10	28.07 ± 3.91	20.20 - 36.64
4	0.73 ± .10	29.00 ± 3.97	21.14 - 37.68
5	0.93 ± .11	36.70 ± 4.33	28.67 - 46.46
6	0.58 ± .09	22.84 ± 3.59	14.79 - 30.97
7	1.10 ± .12	43.26 ± 4.68	34.83 - 54.25
8	1.38 ± .14	54.37 ± 5.46	44.79 - 67.83
9	1.12 ± .12	44.08 ± 4.75	35.58 - 55.24
10	1.42 ± .14	56.09 ± 5.57	46.30 - 69.99
11	1.73 ± .17	68.36 ± 6.55	56.86 - 85.42
12	0.73 ± .11	35.34 ± 5.18	25.88 - 49.25
13	0.46 ± .09	22.82 ± 4.54	9.79 - 33.16
14	0.93 ± .11	45.64 ± 5.43	37.63 - 63.49
15	0.72 ± .10	35.71 ± 4.79	26.03 - 49.74
16	1.12 ± .11	55.18 ± 5.63	48.16 - 77.22
17	1.51 ± .15	74.66 ± 7.16	68.53 -106.46
18	1.05 ± .11	51.74 ± 5.47	44.39 - 72.18
19	0.58 ± .09	28.80 ± 4.59	17.05 - 40.22
20	1.04 ± .11	51.13 ± 5.43	43.74 - 71.31

[1]Results based on 2 HCG augmentation bioassays and expressed as a ratio (R) of test to standard regression lines (± S. D.).

[2]Micrograms (± S. D.) of NIH-FSH-S_1 equivalent per mg. dry pituitary.

Table XV

PLASMA FOLLICLE STIMULATING HORMONE RELATIVE POTENCIES FOR SUCCESSIVE DAYS OF THE ESTROUS CYCLE IN GILTS

Day of Cycle	Slope Ratio (R)[1]	Mean Relative Potency[2]	95% Fiducial Limits
1	1.11 ± .34	34.70 ± 10.60	22.27 - 56.92
2	1.71 ± .46	52.70 ± 14.15	37.35 - 88.25
3	1.45 ± .40	44.50 ± 12.40	30.88 - 73.73
4	1.06 ± .33	32.50 ± 10.15	20.34 - 53.35
5	1.92 ± .51	61.60 ± 16.29	41.47 -106.77
6	1.23 ± .36	37.70 ± 11.11	25.18 - 61.96
7	1.56 ± .43	48.10 ± 13.14	33.76 - 80.06
8	1.15 ± .35	36.00 ± 10.85	23.39 - 59.04
9	1.48 ± .41	44.00 ± 12.18	30.59 - 72.96
10	1.58 ± .43	48.60 ± 13.26	34.10 - 80.89
11	1.67 ± .45	54.50 ± 14.73	38.53 - 91.32
12	1.61 ± .44	48.70 ± 13.22	34.31 - 81.27
13	1.77 ± .47	56.50 ± 15.10	40.16 - 94.97
14	1.56 ± .43	50.80 ± 13.94	35.71 - 84.66
15	1.73 ± .47	50.40 ± 14.88	39.32 - 93.12
16	1.70 ± .46	54.34 ± 14.62	38.53 - 91.05
17	1.66 ± .45	53.30 ± 14.40	37.65 - 89.07
18	1.93 ± .51	68.60 ± 18.10	48.98 -115.96
19	3.21 ± .82	125.30 ± 31.96	89.59 -219.94
20	2.59 ± .67	78.20 ± 20.11	56.18 -135.44

[1]Results based on one HCG augmentation bioassay and expressed as a ratio (R) of test to standard regression lines (± S. D.)

[2]Micrograms (± S. D.) of NIH-FSH-S_1 equivalent per 10 ml. plasma.

Table XVI

ANALYSIS OF VARIANCE FOR HCG-AUGMENTATION BIOASSAYS USED TO EVALUATE DRY ANTERIOR PITUITARY FSH CONCENTRATIONS FOR SUCCESSIVE DAYS OF THE ESTROUS CYCLE IN GILTS

Source of Variation[1]	df	Sums of Squares	Mean Squares	F Ratio
Total	102	2175023.50		
Mean	1	1937661.00	1937661.00	
Regression	8	177888.20	22236.03	35.35**
Blanks	1	1495.13	1495.13	2.38
Intercept	7	4508.00	644.00	1.02
Error	85	53471.27	629.07	

[1]Bioassay SP-3 (days 1 through 11 of pituitary)

**($P < .01$)

Table XVI (continued)

Source of Variation[1]	df	Sums of Squares	Mean Squares	F Ratio
Total	175	9481907.90		
Mean	1	8296569.00	8296569.00	
Regression	12	885521.08	73793.42	42.50**
Blanks	1	159.00	159.00	0.08
Intercept	11	39235.10	3566.83	2.05
Error	150	260423.68	1736.16	

[1]Bioassay SP-4 (days 12 through 20 of pituitary)

**($P < .01$)

Table XVII

ANALYSIS OF VARIANCE FOR HCG-AUGMENTATION USED TO EVALUATE PLASMA FSH CONCENTRATIONS DURING THE ESTROUS CYCLE IN GILTS

Source of Variation[1]	df	Sums of Squares	Mean Square	F Ratio
Total	287	1976181.10		
Mean	1	1897176.10	1897176.10	
Regression	20	36448.90	1822.45	11.19**
Blanks	1	7.88	7.88	0.05
Intercept	19	1812.80	95.41	0.59
Error	246	40062.00	162.85	

[1]Bioassay SP-2 (days 1 through 20 of plasma)

**($P < .01$)

Appendix C

Table XVIII

Liquid Scintillation and Gas Liquid Chromatographic Data for Extracted Luteal Tissue and Daily Luteal Progesterone Concentrations During the Estrous Cycle in Gilts

Estrous[1] cycle day	Mean[2] (CPM)	Background corrected (CPM)	Progesterone by GLC (ug.)	Per cent recovery[3]	Luteal progesterone[4]
1	425.2	366.3	trace	61.3	trace
2	399.3	340.4	trace	56.8	trace
3	195.8	136.9	1.1	22.8	39.4
4	283.0	224.1	5.5	37.3	37.9
5	256.3	197.4	3.9	32.8	26.6
6	267.1	209.2	5.6	35.8	38.3
7	216.5	157.6	4.4	26.2	31.3
8	247.3	188.4	3.7	31.4	42.5
9	101.9	43.0	1.3	7.2	20.8
10	110.6	51.7	3.3	8.6	49.8
11	244.5	185.6	7.9	30.8	43.7
12	221.9	163.0	15.8	27.1	76.7
13	316.6	257.7	17.2	42.8	53.5
14	152.0	93.1	2.8	27.2	27.2
15	205.3	146.4	7.3	24.3	57.5
16	166.1	107.2	2.7	17.8	35.4
17	207.4	148.5	2.6	24.7	15.3
18	157.3	98.4	trace	16.3	trace
19	206.8	147.9	0.7	24.6	0.5
20	149.5	90.6	0.4	15.0	0.9
Standard	6067.9	6009.0			
Blank	58.9				

[1]Standard is 7α-^{3}H-progesterone.

[2]22 X 10^{-3} uc (0.1 ml.) of 7a-^{3}H-progesterone added per sample before extraction and 0.1 of extracted sample counted.

[3]Mean recovery = 28.54%.

[4]Micrograms per gm. of wet luteal tissue.

Appendix D

Parallel - Line Assay Potency Calculation

An example calculation of LH relative mean potency and the 95% fiducial limits are outlined from the ovarian ascorbic acid depletion assay data in part three of this experiment. The procedure illustrated below was based on specific data taken from bioassay 01A (Table X) involving the analysis of day 1 pooled anterior pituitary tissue from 3 gilts. The following steps were followed in each potency calculation of the OAAD bioassays:

1. Assay Data[1]

Preparations	Total Low Dose	Total High Dose	Total High + Low	High - Low	No. Assay Rats
Standards ($NIH-LH-B_4$)	462.1	277.5	739.6	-184.6	10
test (Day 1)	351.8	179.2	531.0	-172.6	10
total	813.9	456.7	1270.6	-357.2	20

[1]Data expressed in ug. of ascorbic acid per 100 mg. rat ovary.

2. Analysis of Variance:

Source of Variation	df	Sums of Squares	Mean Square	S
Total	19	11380.36		
Preparations	1	3175.70	3175.70	
Regression	1	6379.59	6379.59	
Parallelism	1	7.20	7.20	
Error	16	2817.87	176.12	13.27

3. Average Regression Coefficient:

$$b_s = \frac{\text{High-Low}}{\text{Error df}} = \frac{-357.2}{16} = -22.32$$

$$V(b) = \frac{\text{Error Mean Square}}{\text{Error df}} = \frac{176.12}{16} = 11.01$$

$$g = \frac{V(b)\ (t)^2_{(.975).(6)}}{(b_s)^2} = \frac{(11.01)\ (2.12)^2}{(-22.32)^2}$$

$$g = .099$$

4. Calculation of Log_{10} relative mean potency (m):

$$\frac{\bar{y}\ [\text{Day } 1] - \bar{y}\ [\text{standard}]}{\bar{n}} = \frac{531.0 - 739.6}{10} = -20.86$$

$$M = \bar{x}_s - \bar{x}_t \pm \frac{\bar{y}t - \bar{y}_s}{b_s}$$

$$M = 0 - \frac{-20.86}{-22.32}$$

$$M = 0.9346$$

5. Calculation of Fiducial Limits of (m):

$$M_L\ M_u = \frac{\pm\ [\bar{M} \pm \frac{t}{b_s}\ [1-g(\frac{1}{\bar{n}_s} + \frac{1}{\bar{n}_r}) \pm \frac{m^2}{\text{Error df}}]\ s^2}{1-g}$$

$$M_L\ M_u = \frac{.9346 + .095\ \ [(.911)\ (.2) + .0546]\ \ 176.12}{.911}$$

$M_L = 0.3523$ $\qquad$ $M_u = 1.6996$

6. Calculation of Relative Potency (R):

$$R = \frac{X_s}{X_t} \text{ antilog } M\ (1/2 \log_{10} 2)$$

$$= \frac{5 \text{ ug.}}{5 \text{ mg.}} \text{ antilog } (.9346) \times (.1505)$$

$$= (1 \text{ ug./mg.}) \text{ antilog } .1407$$

$= 1.383$ ug./mg. of dry Pituitary (NIH-LH-B_4)

$= \frac{1.383}{.94} = 1.471$ ug./mg. dry pituitary (NIH-LH-S_1 equivalent)

$R_L = (1 \text{ ug./mg.}) \text{ antilog } (.3523) \times (.1505)$

$= \text{antilog } .053$

$= 1.130$ ug./mg. dry pituitary (NIH-LH-B_4)

$= \frac{1.130}{.94} = 1.383$ ug./mg. dry pituitary (NIH-LH-S_1 equivalent)

$R_u = (1 \text{ ug./ml.}) \text{ antilog } (1.6996) \times (.1505)$

$= \text{antilog } .2558$

$= 1.801$ jg./mg. dry pituitary (NIH-LH-B_4)

$= \frac{1.801}{.94} = 1.916$ ug./mg. (NIH-LH-S_1 equivalent)

Slope - Ratio Assay Potency Calculation

An example calculation of FSH relative mean potency and the 95% fiducial limits are outlined from the HCG-augmentation assay data in part three of this experiment. The procedure illustrated below was based on specific data taken from bioassay SP-3 (Table XIV) involving the analysis of day 12 pooled anterior pituitary tissue from 4 gilts. The following steps were followed in each potency calculation of the slope-ratio bioassays:

1. Slope-ratio of test preparation regression line, to standare preparation regression line:

$$R = \frac{b_t}{b_s} = \frac{42.54}{59.40} = .7162$$

$$\text{Mean Relative Potency} = (.7162) \times \frac{(150 \text{ ug.})}{4 \text{ mg.}}$$
$$= (.7162) \times (37.5 \text{ jg./mg.})$$
$$= 26.86 \text{ ug./mg. (NIH-FSH-}P_1\text{)}$$
$$= \frac{26.86}{.76} = 35.34 \text{ ug./mg. (NIH-FSH-}S_1\text{ equivalent)}$$

$$N = (7) \times (102)$$

$$t = 1.989$$

$$g = \frac{64t^2 \, S^2}{7 \, N \, b_s^2} = \frac{(64)\,(1.989)^2\,(25.09)}{(714)\,(59.4)^2} = .2148$$

2. Calculation of Fiducial Limits:

$$R_L\, R_u = \frac{\left[R - \frac{9g}{16} \pm \frac{t}{b_s} \quad \frac{85^2}{7N} \quad (8-9R + 8R^2 - \frac{175g}{32}) \right]}{1-g}$$

$$R_L\, R_u = \frac{.7162 - 1.208 + \quad .0335 \quad 7.048(8-6.446 + 4.103 - 1.175)}{.7852}$$

$$R_L\, R_u = \frac{.5954 \pm 1.883}{.7852}$$

$$R_L = .5185 \qquad R_u = .9981$$

Low Relative Potency = (.5185) x (37.5 ug./mg.)

= 19.44 ug./mg. (NIH-FSH-P_1)

= $\frac{19.44}{.76}$ = 25.58 ug./mg. (NIH-FSH-S_1 equivalent)

High Relative Potency = (.9981) x (37.5 ug./mg.)

= 37.43 ug./mg. (NIH-FSH-P_1)

= $\frac{37.43}{.76}$ = 49.25 ug./mg. (NIH-FSH-S_1 equivalent)

3. Calculation of Slope Ratio (R) Standard Error

$$S_R^2 = \frac{s^2}{b_s^2} [C_{ss} - 2 R C_{st} + R^2 C_{tt}]$$

$$S_R^2 = \frac{629.07}{(59.4)^2} [.0564 - 2R(.0231) + R^2 (.0564)]$$

$$S_R = .105$$

Relative Potency = (.105) x (37.5 ug./mg.

= 3.94 ug./mg. (NIH-FSH-P_1)

= $\frac{3.94}{.76}$ = 5.18 ug./mg. (NIH-FSH-S_1 equivalent)

VITA

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Candidate for the Degree of

Doctor of Philosophy

Thesis: SOME TEMPORAL RELATIONSHIPS BETWEEN THE ANTERIOR PITUITARY CORPORA LUTEA AND ENDOMETRIUM OF NULLIPAROUS GILTS DURING THE ESTROUS CYCLE

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