PLASMA CONCENTRATIONS OF LUTEINIZING HORMONE, FOLLICLE STIMULATING HORMONE AND PROLACTIN IN OVARIECTOMIZED, HYSTERECTOMIZED AND INTACT SWINE

by
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ABSTRACT

Title of Thesis: Plasma Concentrations of Luteinizing Hormone, Follicle Stimulating Hormone and Prolactin in Ovariectomized, Hysterectomized and Intact Swine

William Walter Wilfinger, Jr., Doctor of Philosophy, 1974

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In order to critically evaluate the temporal patterns of plasma luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL); blood samples were collected daily at 0600, 1200, 1800 and 2400 hrs from intact, sham, ovariectomized and hysterectomized Yorkshire sows via indwelling vena caval cannulae. The maximum preovulatory plasma LH concentration in the intact and sham operated swine (mean ± 1 SD, 7.06±2.40 ng/ml) was reached between 2400 hrs on the first day of estrus (day 0) and 0600 hrs on day 1. Preovulatory LH peaks averaged 28.75±4.76 hrs duration from initial rise from, and return to, a baseline concentration of 1.19±.25 ng/ml plasma. In addition to the preovulatory LH peak, an average of 8.75 luteal phase peaks was observed during each estrous cycle. Approximately 69.5% of the luteal phase peaks had an average duration of 12.65±1.79 hrs, an average maximum plasma concentration of 2.29±.55 ng/ml and occurred during days 2 to 17. The remaining luteal phase peaks reached a maximum concentration of 2.86±1.04 ng/ml and occurred during days 2 to 13. The average duration of these luteal phase peaks was 18.61± 3.49 hrs.

The largest and most consistent plasma FSH peak in the intact and sham operated swine occurred between days 2 and 3. This postovulatory peak had a duration of 58.17±16.25 hrs and reached an average maximum concentration of 13.14±9.42 ng/ml before returning to a baseline of 4.76± 1.56 ng/ml plasma. The initial rise of the postovulatory FSH peak coin-

cided with the initial rise of the preovulatory LH peak, but did not reach maximum concentration until 2.66±.45 days after the preovulatory LH peak had reached maximum concentration. In some animals, a luteal phase FSH peak occurred between days 8 to 14. The luteal phase FSH peaks had a duration of 18.09±5.36 hrs and an average maximum concentration of 9.76± 3.78 ng/ml plasma.

An average of 2.58±1.08 plasma PRL peaks was observed between day 0 and day 2 of the estrous cycle in the intact and sham operated swine. These peaks averaged 20.54±4.02 hrs in duration and reached maximum concentrations of 11.49±2.97 ng/ml before returning to a baseline of 6.54± 1.29 ng/ml plasma. They reached their maximum concentration approximately 9.12±9.84 hrs after the maximum concentration of the preovulatory LH peak had been reached. Larger prolactin peaks occurred during the follicular phase of the estrous cycle. An average of 3.09±1.38 peaks occurred between days 14 and 19. These peaks had an average duration of 18.98±3.30 hrs and reached a maximum concentration of 14.28±4.27 ng/ml plasma.

Shortly after removal of the ovaries (6 to 8 hrs), baseline LH (2.2± .14 ng/ml) and FSH (12.20±2.90 ng/ml) plasma concentrations in the ovariectomized swine were significantly greater ($P<.05$) than those found in the intact and sham operated animals, but baseline prolactin concentrations (6.70±2.10 ng/ml) were not significantly different ($P>.05$) from the controls. The temporal patterns of maximum plasma concentrations associated with LH (3.47±.21 ng/ml), FSH (21.43±.82 ng/ml) and PRL (9.32±2.00 ng/ml) peaks were not similar among the ovariectomized animals.

Baseline LH (1.12±.34 ng/ml), FSH (5.30±2.09 ng/ml) and PRL (6.05± 1.89 ng/ml) concentrations in the hysterectomized swine were not significantly different ($P>.05$) from the baseline concentrations in the intact and sham operated animals. Numerous plasma LH peaks (14.30±4.59) were

observed during the 20-day collection period. These peaks averaged 19.50 ±1.49 hrs in duration and reached maximum concentrations of 2.63±.23 ng/ml before returning to baseline. Plasma FSH and PRL peaks averaged 21.00 ±4.51 and 17.69±3.50 hrs in duration and reached maximum concentrations of 8.12±1.95 and 10.18±2.95 ng/ml before returning to baseline.

Mean plasma LH concentrations in the intact, sham, ovariectomized and hysterectomized swine were significantly greater at the 2400 hr collections than at the 1800 hr collections.

A critical assessment of plasma hormone concentrations is an essential prerequisite to a basic understanding of the physiological mechanisms governing the reproductive process. The descriptive data provided by this study will enable future investigators to design critical experiments to evaluate the physiological importance of these hormone fluctuations, thereby broadening our basic understanding of the mechanisms which govern the reproductive cycle.

DEDICATION

To my Father and Mother for their

countless contributions to

my life and education

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I. INTRODUCTION

Relatively little is known about the temporal fluctuations of plasma gonadotropins in the major domestic animals. The inability of investigators to develop accurate and precise radioimmunoassays (RIA) which are capable of quantifying luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL) in any one of the domestic species has prevented a critical evaluation of gonadotropin concentrations during the estrous cycle. Due to the scarcity of direct measurements of plasma gonadotropin concentrations, many of the conclusions regarding the interaction of gonadotropins and their control have been made by inference, and in most cases cannot adequately explain hormonal and morphological changes in the ovary.

The primary intent of this research project was to critically evaluate gonadotropin concentrations in the peripheral plasma of intact and treated domestic swine. The 2,000 plasma samples collected from the 24 Yorkshire sows used in this study have provided additional information to further the understanding of the physiological mechanisms governing the temporal fluctuations of plasma LH, FSH and PRL in the intact, ovariectomized and hysterectomized pig.

II. LITERATURE REVIEW

A. Morphological Changes in the Ovary During the Estrous Cycle of the Pig

The domestic pig reaches sexual maturity between 6 and 7 months of age, at which time sexual competence is characterized by the animal's ability to produce fertile ova and maintain pregnancy until term. Maturity is characterized by the recurrence of periods of sexual activity ("heat" or "estrus") at intervals of approximately 21 days. The pig is unique among the large domestic animals in being polytocous. It is also polyestrous and will breed at any time of the year.

The growth patterns of the ovarian follicle in the pig, sheep and cow have been reviewed by Robertson (1969). In most of the studies cited by Robertson (1969), follicular development has been deduced indirectly from observations on the number and size of the follicles present in the ovaries of animals killed at different stages of the estrous cycle. The classical interpretation of these data suggests that porcine, ovine and bovine follicles enlarge rapidly during the first few days of the cycle and then remain fairly constant in size during the luteal phase before commencing to increase again some time prior to ovulation. However, detailed quantitative studies in the cow (Rajakoski, 1960; Dufour et al., 1972), pig (Green, 1950) and sheep (Smeaton and Robertson, 1971) support the hypothesis that successive waves of follicular growth and atresia occur during the luteal phase of the estrous cycle and only the follicles which enlarge during the 2 days before estrus ovulate and form corpora lutea.

In a study of the grossly visible follicles of 58 sows slaughtered at timed intervals during the estrous cycle, Green (1950) observed only

a few follicles of nonovulatory size present on the ovary on day 0 (first day of heat). By day 1 only the "chosen follicles" are present; however, ovulation between days 1 and 2 results in the complete absence of follicles by the end of day 2. By the fourth day of the cycle, follicular growth has begun and the number of small follicles and follicles 6 to 7 mm in diameter increase rapidly from the fourth to the tenth day. On the ninth day and again on the eleventh day there is a sudden increase in the number of follicles over 7 mm in diameter, but these large follicles disappear. From day 11 until day 17, there is an increase in the number of follicles of the 6 and 7 mm group; however, by the end of day 17 they have once again decreased in number. The regularity with which smaller follicles decrease in numbers when the next largest size becomes more numerous is noteworthy.

Ovulation occurs approximately 36 hrs after the onset of estrus. The passage of the ova through the oviduct takes 3 days. If copulation and fertilization has occurred, segmentation of the zygote to the 2, 4 or 6 blastomere stage will occur during the passage of the zygote through the oviduct (Corner, 1921). About 4 days after ovulation, the unfertilized ova enter the uterus and degenerate. However, they do not disappear completely until at least the seventh or eighth day after ovulation. The embryos entering the uterus will continue to develop in utero, undergoing implantation between days 10 to 15 (Corner, 1921).

The extrusion of the ovum from the graafian follicle during ovulation allows the luteinization and vascularization of the granulosa layer to proceed. The newly formed corpus luteum (CL) will continue to hypertrophy until a diameter of 8 or 9 mm is reached by the end of one week's growth. Seven days after ovulation the corpus luteum is usually smeared and its cells

have become fully differentiated. The gross morphology of the CL remains relatively constant until day 13 when the luteal cells show a sudden increase in diameter and the appearance of fibrils (Green, 1950).

Cavazos et al. (1969) have characterized the granulosa cells of the corpus luteum in gilts by electron microscopy. Cytoplasmic vesicles were observed on days 8 and 12 of the cycle. These vesicles are believed to be related to protein uptake and have been associated with the transport of absorbed protein to lysosomes. In the luteal cell, many coated vesicles appear during maximum progesterone secretion (days 8 through 12). Cavazos et al. (1969) have hypothesized that these vesicles contain hydrolases and may be the first indication of eventual regression and cytolysis which occur in the CL during the normal estrous cycle.

Day 13 is considered to mark the end of the growth of the CL and the beginning of their visible degeneration (Green, 1950). Within 2 or 3 days the CL decrease from 8.5 or 10 mm to 6 mm and their color changes from pink to the whitish tone of scar tissue. The luteal cells become laden with lipid, contain numerous membrane-bound lysosomes and are separated by vast amounts of connective tissue. As regression continues, the lutein cells disappear and the CL are transformed into corpora albicantia. If pregnancy occurs, the CL undergo further growth until the maximum size of 10 to 11 mm is reached 2 or 3 weeks later. These CL remain functional and support pregnancy throughout the 112 day gestation period of the pig.

With the demise of the CL, follicular growth accelerates over a period of 3 to 6 days until ovulable follicles of 7 to 10 mm are present on the ovary. When the follicles have reached "maturity", their steroidal secretions initiate estrus and the renewal of the cyclic reproductive process.

B. Hypophyseal and Plasma Concentrations of Gonadotropins During the Estrous Cycle of the Pig and Other Species

Using a "total gonadotropin" bioassay (increase in chick testes weight) Robinson and Nalbandov (1951) showed that gonadotropin potency was lowest during the 2 days of heat, and remained low after ovulation until the seventh day of the cycle when it increased suddenly and remained high until day 19. Parlow et al. (1964) measured the concentration of FSH and LH in the anterior lobe of the pituitary gland (hereafter called "pituitary") of young gilts during the estrous cycle. Using more sensitive bio-assay methods (HCG-augmentation method for FSH and ovarian ascorbic acid depletion method for LH) they found the concentration of both hormones to be low at estrus and remain low until the third day of the estrous cycle. By the ninth day pituitary content had doubled, and by the seventeenth day the concentrations had increased by approximately three fold. An increase in the secretion of pituitary FSH and LH during days 3 to 9 and days 17 to 0 may serve as the stimulus for the accelerated follicular growth observed at this time of the estrous cycle.

The first measurements of LH in the serum of gilts were determined from large pools of blood from which LH was extracted and bioassayed by the ovarian ascorbic acid depletion bioassay (Anderson and McShan, 1966). These authors determined venous LH concentration to be 100 ng of NIH-LH-S1/ml of plasma 24 hrs after the onset of estrus, while the plasma concentrations on the thirteenth and nineteenth days were 1 to 3 ng/ml of plasma. Prokofiev (1968), using a similar method for concentrating and measuring serum gonadotropins, showed that at the beginning of estrus LH concentrations were 72 to 90 ng NIH-LH-S_5/ml and rose to a peak level of 115 to 420 ng/ml, 6 to 12 hrs after the onset of estrus. This high level

was maintained for 2 to 3 days (until days 3 and 4) before the concentration decreased to 20 to 80 ng/ml. Liptrap and Raeside (1966), using the ovarian cholesterol depletion method for measuring LH, reported elevations of LH activity coinciding with a peak of urinary estrogen excretion occurring 40 to 48 hrs before the time of ovulation.

After the development of specific RIA for porcine LH (Niswender et al., 1970; Rayford et al., 1971), LH concentrations were found to be considerably less than had previously been reported. Niswender et al. (1970), measured LH during estrus (1.5 to 5 ng/ml NIH-LH-S1), and found the serum concentrations in the pig to rise during or slightly before estrus, remaining elevated for approximately 24 hrs. Rayford et al. (1971), Tillson et al. (1970) and Henricks et al. (1972) confirmed the observation of Niswender et al. (1970) that circulating concentrations of LH were highest during estrus while gradually decreasing during the remainder of the cycle.

The magnitude of the LH peak in other domestic animals is quite variable. LH concentrations in the ewe have been reported to rise 0 to 12 hrs after the onset of heat and to reach maximum concentrations of 25 to 460 ng/ml over a time period of about 12 hrs (Geschwind and Dewey, 1968; Niswender et al., 1968; Goding et al., 1969; Wheatley and Radford, 1969; Scaramuzzi et al., 1970). The basal LH concentrations ranged from .2 to 2 ng/ml, sometimes reaching concentrations as high as 5 to 12 ng/ml; however, some of this variation may be due to a secondary peak at day 14 (Scaramuzzi et al., 1970). Bovine LH reaches a maximum concentration approximately 4 to 6 hrs after the onset of heat (7 to 60 ng/ml) and remains elevated for 10 to 20 hrs (Schams and Karg, 1969; Niswender et al., 1969; Henricks et al., 1970; Snook et al., 1971). Luteal phase LH concentrations ranged from .4 to 4 ng/ml with peaks occurring between days 7 and 8 (2.3

to 9 ng/ml) and 4 to 7 days before ovulation (3.5 to 7.8 ng/ml) (Schams and Karg, 1969; Henricks et al., 1970; Snook et al., 1971).

In contrast to most other species, including sheep and cattle, the magnitude of the preovulatory LH peak in the pig, relative to LH concentrations during other stages of the cycle, is relatively small (2 to 5 times greater than luteal phase LH concentrations). It appears that the sow ovary is extremely sensitive to LH or perhaps the sow is more sensitive to handling during estrus which may suppress normal ovulatory LH concentrations (Geschwind, 1972). The occurrence of an LH peak on the day of estrus in gilts is consistent with observations in the sheep and cow; however, luteal phase LH fluctuations have not been observed in the pig.

A detailed study of serum FSH concentrations in the pig has not been reported. However, pituitary FSH content has been measured in the pig, cow and sheep. Parlow (1964) found the mean concentration of FSH in the anterior pituitary to be low at estrus (day 0) and remain low until day 3. Between days 3 and 9 a sharp increase in FSH pituitary content was noted. This elevated level of FSH was maintained through day 17 and then declined until day 0 of the next cycle. Parlow (1964) associated these changes with increased follicular growth during days 3 to 7 and 17 to day 0. It was also hypothesized that an increase in pituitary FSH content between days 3 to 15 was the result of progesterone inhibition of FSH release which allowed the synthesis and accumulation of the hormone. The regression of the corpora lutea at approximately day 15 results in a decrease in plasma progesterone concentration and a concomitant decrease in pituitary FSH. The data from this study (Parlow, 1964) failed to reveal any change in the FSH:LH ratio at estrus or at any of the other stages of the cycle.

Short et al. (1968) reported pituitary FSH concentration to be suppressed between days 1 and 7, increase dramatically (4 times) between days 7 to 13 and then again decline between days 13 and 19. Parlow et al. (1964) noted a dramatic increase in pituitary FSH content between days 3 to 9; however, Short et al. (1968) found FSH content to be low at day 7. These separate observations support the hypothesis that pituitary FSH concentrations increase dramatically between day 7 and 9, concomitant with increased progesterone output by the corpora lutea.

Robertson and Rakha (1966) found ovine pituitary FSH content decreasing 8 hrs before the onset of estrus, at which time 28% of the FSH content of the pituitary had been released. FSH discharge continued at a steady rate until 6 hrs after the onset of estrus and resulted in an additional 24% decrease in hypophyseal FSH. Since LH release did not begin until 8 hrs after the onset of estrus, the release of FSH and LH from the pituitary was thought to be asynchronous. The FSH released before estrus was suspected of being the gonadotropin responsible for stimulating the production and secretion of the steroid hormones which induce behavioral estrus. Dierschke and Clegg (1968) assayed cavernous sinus blood at designated times after declared estrus. Their data demonstrated a maximal release of both pituitary LH and FSH during the interval 8 to 16 hrs after the onset of estrus. A drop of 33% in the total FSH content per gland after declared estrus was similar to that reported by Robertson and Rakha (1966).

Bjersing et al. (1972) and L'Hermite et al. (1972) have measured ovine FSH by RIA and have found concomitant drops of both serum LH and FSH shortly after estrus. L'Hermite et al. (1972) measured serum FSH at 4 hr intervals from -6 days before estrus until 2 days after estrus. Serum concen-

trations remained fairly constant between days -6 to -4 (122 ng/ml NIH-FSH-S_4), began to decrease from day -4 to 0 (110 ng/ml) and showed an ovulatory peak on day 0 (150 ng/ml). The peak lasted for about 12 hrs before serum concentrations returned to 110 ng/ml. However, serum concentrations once again began to rise and reached maximum concentrations early on day 1. Ovulation in sheep occurs approximately 24 hrs after the ovulatory surge of LH. It is interesting to note that the second FSH peak occurring on day 1 followed the ovulatory LH surge by 24 hrs. The low level of progesterone and estrogen observed in the ovarian venus effluent at this time may stimulate FSH synthesis and secretion, thus providing the stimulus for rapid follicular growth observed early in the ovine estrous cycle.

Rakha and Robertson (1965) also found a decrease in LH and FSH in the bovine pituitary between the onset and the end of estrus. In an excellent study by Hackett and Hafs (1969) a 49% decrease in bovine pituitary FSH content was observed between days 18 and 20 while another 46% reduction occurred by day 0 accounting for a 73% loss from day 18 to day 0. FSH content remained low at day 4, but increased continuously from day 4 to day 18. These data suggest that pituitary FSH may be released during the first half of the estrous cycle concomitant with the first wave of follicular growth (Rajakoski, 1960).

Sensitive radioimmunoassays for ovine and bovine prolactin have been developed recently (Reeves and Arimura, 1970; Davis et al., 1971; Raud and Odell, 1971; Swanson and Hafs, 1971). Reeves and Arimura (1970) reported ovine serum prolactin concentrations to be significantly higher during proestrus (49 ng/ml) and estrus (day 0) than during the second day of estrus (11 ng/ml), metestrus (15 ng/ml) or diestrus (13 ng/ml).

Elevated serum prolactin concentrations during proestrus and early estrus were also observed by Davis et al. (1971). In addition to the preovulatory prolactin surge, recurring prolactin peaks were observed every 3 to 4 days during the ovine estrous cycle. The available data dealing with plasma prolactin concentrations in the cow is far more sketchy. However, Raud and Odell (1971) and Swanson and Hafs (1971) have observed elevated prolactin concentrations during estrus. The limited amount of data obtained by use of the pigeon crop gland assay suggests that porcine pituitary prolactin content is lowest during estrus, with increased accumulation during the luteal phase until days 18 to 19 (Day et al., 1959; Anderson et al., 1972; Threlfall et al., 1972).

Although radioimmunoassays for porcine prolactin have appeared in the literature (Raud and Odell, 1971; Brinkley et al., 1972), no studies of prolactin concentrations during the porcine estrous cycle have been reported.

C. Plasma and Urinary Concentrations of Gonadal Steroids During the Estrous Cycle of the Pig and Other Species

Porcine plasma progesterone concentrations have recently been measured by Stabenfeldt et al. (1969a), Edqvist and Lamm (1971) and Henricks et al. (1972). These authors found progesterone concentrations to increase steadily between days 2 and 7. After day 7 a number of animals began to show a plateau in progesterone concentration which fluctuated between 20 and 30 ng/ml. This plateau continued for 3 to 7 days and was followed by a further increase during days 13 and 14 where a peak of 28 to 50 ng/ml was attained. Two days after peak progesterone concentrations had been observed, the concentration had dropped to less than 1 ng/ml. A deviation in the linear increase of plasma progesterone concnetration at or around day 7

occurred at approximately the same time the corpora lutea had completed luteinization. It is interesting to note that similar patterns of plasma progesterone have also been observed in cattle (Henricks et al., 1970; Snook et al., 1971) and sheep (Thorburn et al., 1969; Stabenfeldt et al., 1969b).

The first reports of estrogen determinations during the porcine estrous cycle were made by Velle (1959), Lunaas (1962), Raeside (1963) and Bowermann et al. (1964). Measuring urinary estrone, the principal estrogen in swine, they found estrone concentrations to be elevated 2 or 3 days before estrus, reaching maximum concentrations of 1.8 to 20.5 ng/liter of urine prior to or coincident with estrus. Frequently, there was a percipitous decline in estrogen excretion after the onset of estrus. Lunaas (1962) also reported fluctuations in urinary estrone concentrations during days 7 and 8 and occasionally during days 11 or 16.

Recently, Henricks et al. (1972) reported plasma estrogen concentrations to rise several days prior to estrus, reaching maximum concentration (60 to 70 ng/ml) on the day of estrus. Plasma estrogen concentrations did not begin to increase rapidly until progesterone concentrations had begun to fall. Henricks et al. (1972) also reported a minor estrogen peak (20 to 30 pg/ml) between days 4 and 8 in all 6 pigs used in their study.

In the sheep, the ovulatory surge of LH is preceded by a rise in plasma estrogens. The earliest detectable rise occurred about 24 hrs before the rise of LH concentration. In addition to the ovulatory estrogen peak (650 to 900 pg/ml), a smaller peak was observed on day 2 (~250 pg/ml) (Scaramuzzi et al., 1970; Cox et al., 1971) and day 8 (300 pg/ml). These peaks were not statistically significant and their importance must await further evaluation.

D. Summary

Little is known about the mechanisms which control the ovarian subsystem in the pig. Extensive research in the rat and other species has shown that steroids which are produced in the ovary are capable of influencing the release of hypothalamic hormones (releasing factors) which in turn stimulate or inhibit the synthesis or secretion of pituitary hormones. Due to the vast amounts of data which have accumulated over many years, the concept of ovarian "feedback" has become one of the basic theories of present day endocrinology. All current theories of ovarian control incorporate the feedback principal in some way. Since simultaneous temporal correlations between the gonadotropins and ovarian steroids have not been reported, little direct evidence exists to define what hormone concentrations are required to regulate the estrous cycle.

The limited amount of data related to hormone concentrations during the porcine estrous cycle are summarized in table 1. In general, pituitary LH, FSH and PRL content follows a cyclic pattern during the estrous cycle. There is a significant depletion of pituitary gonadotropin content coincident with behavioral estrus. Pituitary content remains relatively low for several days and eventually reaches maximum concentrations just prior to the next ovulatory release. It is thought that elevated luteal phase progesterone concentrations inhibit hormone release causing increased accumulation of hormone within the pituitary gland (Parlow et al., 1964). During the follicular phase, pituitary content is elevated and the eventual hormone release during estrus is thought to be due to the stimulatory effects of elevated preovulatory serum estrogen concentrations (Sawyer, 1964).

The importance of the simultaneous decrease in LH, FSH and PRL pituitary

content during ovulation must await clarification. Although LH acts synergistically with FSH to promote the secretion of estrogen by follicles undergoing maturation, only LH is required to induce ovulation in the pig (du Mesnil du Buisson and Leglise, 1963; Rondell, 1970; Spies et al., 1967). Since prolactin has been found to be ineffective in inducing ovulation or maintaining luteal function in the pig (Sammelwitz and Nalbandov, 1958; Anderson et al., 1967), the relative importance of this hormone during the estrous cycle remains obscure.

Plasma LH concentrations appear to parallel fluctuations in pituitary content since plasma concentrations remain low throughout the estrous cycle, except for the first day of estrus (Niswender et al., 1970; Tillson et al., 1970; Rayford et al., 1971; Henricks et al., 1972). This finding supports the hypothesis that rising progesterone concentrations during the first 15 days of the estrous cycle are capable of suppressing LH release. This conclusion is in keeping with the observations of Brinkley et al. (1964 a,b) and du Mesnil du Buisson and Leglise (1963). These investigators found that formation of the porcine corpus luteum occurs independently of hypophyseal support and once ovulation has occurred no further support is required for a normally functioning corpus luteum.

Porcine FSH and PRL plasma concentrations have not been described in the literature. Parlow et al. (1964) has suggested that low pituitary FSH content between days 3 to 9 may reflect an increased secretion of FSH during this period. This hypothesis is supported by the fact that follicular growth is quite pronounced during the early part of the estrous cycle (Green, 1950; Robinson and Nalbandov, 1951; Parlow et al., 1964). Additional support for this conclusion exists in the finding of small urinary and plasma estrogen peaks between days 4 to 8 (Lunaas, 1962; Henricks

et al., 1972). Thus, it appears that the release of FSH early in the cycle stimulates follicular growth before progesterone levels reach concentrations which suppress gonadotropin release.

With the demise of the corpus luteum between days 14 to 15, estrogen concentrations once again begin to rise (Henricks et al., 1972). A decrease in hypophyseal FSH content prior to estrus is presumably due to the secretion of FSH which is needed to support the rapidly growing follicles found on the ovary just prior to ovulation (Parlow et al., 1964). The elevated estrogen concentrations during the follicular phase, superimposed upon a preconditioning period with progesterone, are thought to be responsible for behavioral heat and the ensuing ovulatory gonadotropin surge (Robertson, 1969).

The hormones discussed in this review are believed to supply the ovarian subsystem with the appropriate stimuli needed to regulate the cyclic nature of the reproductive process. The differences in magnitude and duration of plasma gonadotropin concentrations among the domestic animals may account for the inherent variability in the length of the estrous cycle observed among these species. The simultaneous analysis of LH, FSH and PRL within a single animal, the pig, should help to clarify and advance the understanding of the reproductive process in all animals.

III. GENERAL MATERIALS AND METHODS

A. Animals and Animal Care

Twenty-four nulliparous Yorkshire sows, ranging in age and weight from 7 to 14 months and 115 to 150 kg, were checked for heat daily. The day on which the pig would stand rigid when pressure was applied to the loin area was designated as the first day of estrus (day 0). After displaying at least one estrous cycle length between 18 to 23 days, the animals were moved to special holding stalls (figure 1) in the Large Animal Laboratory at the University of Maryland. The restraining stalls were constructed of 2.54 cm pipe (individual stall dimensions length 1.57 m, width .66 m, height .81 m). The stalls limited the mobility of the sows and tethering was not required. The floor of the stall was constructed of steel mesh plate so the animals could be kept clean during their confinement. Although the individual stalls adjoined one another, the animals were physically separated by wire mesh. Each animal had access to automatic waterers and was individually fed 3 pounds of a balanced diet per day. Throughout the duration of the experiment both temperature and light were controlled. The animals were exposed to a daily 14 hr light-10 hr dark sequence with the light period beginning at 0600 hrs. In order to supply the investigator with adequate illumination during the night collections, the animal room was also equipped with dim red lights which were on continuously.

B. Surgical Procedure

The animals were anesthetized with sodium pentobarbital (50 mg/2.28 kg body wt) and restrained. The neck region was scrubbed with surgical soap and swabbed with 10% iodine solution. Using a sterile hypodermic

Figure 1. Restraining stalls used to house the experimental animals during the 20-day blood collection period.

needle, (12 ga) approximately 20 cm of a 3 m length of polyvinyl tubing (Becton-Dickinson #VX44-6179 i.d. 1.12 mm, o.d. 1.65 mm) containing a heparinized (100 units/ml) physiological saline solution (.9% NaCl) was inserted into the anterior vena cava. The tubing was allowed to run under the skin for approximately 5 cm before it was exteriorized. Medical grade adhesive tape was wrapped around the cannula from the point at which the cannula was exteriorized to a distance of about 50 cm. The first 5 cm of taped tubing was sutured to the skin to immobilize the cannula. The remaining portion of the cannula was loosely attached to a thin piece of elastic (1 cm x 80 cm). One end of the elastic was tied to a suture in the skin above the left or right shoulder and the other end was fastened to the stall. The elastic band allowed the cannula to coil and uncoil depending upon the position of the animal within the stall. This procedure was found to be an effective means of preventing the animal from damaging the cannula. Between collections, the free end of the cannula was connected to a stopcock and immersed in 70% ethanol.

The abdominal surgical techniques used for sham, ovariectomy and hysterectomy have been previously reported by Brinkley and Young (1968).

C. Sample Collection and Storage

Immediately following cannulation, blood samples were collected at 6 hr intervals (beginning at 0600 hrs) from the beginning to the end of the collection period (~30 days). However, blood samples drawn during the first 3 days of blood collection were not included in the analysis of plasma hormone concentrations.

The heparanized saline solution used to maintain patency between collection periods was withdrawn prior to withdrawal of blood. A sterile 12 ml syringe was attached to the stopcock and this syringe was attached to an infusion-withdrawal pump (Harvard Apparatus #600-950V). This

procedure was repeated in 2 adjacent animals and then 10 ml of blood was simultaneously withdrawn from the cannulae of 3 animals at a rate of 4.0 ml/min with the aid of the infusion-withdrawal pump. While the samples were being withdrawn, the next set of cannulae were prepared for blood removal.

After the blood samples had been drawn, the cannulae were rinsed with 5 ml of sterile physiological saline and then refilled with 3 ml of heparinized physiological saline solution (100 units/ml). The order in which the animals were sampled was randomized to reduce sampling bias. Alternating cannula preparation and blood withdrawal reduced the collection period for 12 animals to approximately 30 min. If any difficulty arose during the withdrawal of a blood sample, no further attempt was made to withdraw from that cannula until the remaining animals had been bled.

The blood was placed into a 15 ml conical graduated centrifuge tube and returned to the laboratory where the blood was centrifuged at 500 x g for 20 min. The plasma was aliquoted into 5 shell vials, and stored frozen until assayed.

D. Validation of Sample Collection Procedure

In order to evaluate the effect of continuous blood removal on the cellular elements of the blood, packed cell and total plasma volumes were recorded after each blood collection. After the experiment had been concluded, packed cell volumes were regressed with time to determine if continuous blood removal had an effect on packed cell volume. The effect of treatment on packed cell volume after 23 days of continual blood withdrawal was also examined by means of a randomized-block-analysis of variance (Snedecor and Cochran, 1969).

In addition to monitoring packed cell volumes, red and white blood

cell counts were made periodically. During cannulation, cell counts were made once a week between 1300 and 1600 hrs. Blood samples were obtained from the cannulae after the heparinized saline solution had been removed. Red cell counts were made by diluting the cells with Hayem's fluid and counting them on a standard hemocytometer. A similar procedure was used for white cell counts; however, a gentian violet-3% glacial acetic acid solution was used as a diluting fluid. The effect of treatment and time on red and white cell counts was analyzed by analysis of variance (4 x 2 factorial design, Snedecor and Cochran, 1969).

E. Post-Experiment Examination of Reproductive Organs

After the animals had reached the end of the collection period (~30 days), they were sent to a local slaughter house. An investigation of the cervical and abdominal lymph nodes indicated that they were normal in all animals. After a preliminary examination of the carcasses, the reproductive tracts were removed and returned to the laboratory for further examination.

F. Radioimmunoassays

Plasma LH, FSH and PRL were determined with double antibody radioimmunoassays. The specificity, precision, accuracy and sensitivity for the LH (Rayford et al., 1971), FSH (Rayford et al., 1973) and PRL (Brinkley et al., 1972) radioimmunoassays have been reported. The reproducibility for each of the three hormone assays used in this study was estimated by calculating within and between assay variation (table 2).

Purified hormone preparations were labelled with ^{125}I (New England Nuclear NEZ-033H) to specific activities of 60-100 μc/μ gm by the method of Greenwood et al. (1963). All plasma unknowns for a given animal were analyzed in duplicate within a single assay. After completion of a 5-day incu-

bation at 10C (LH and FSH) or a 3 hr incubation at 37C followed by an 18 hr incubation at 10C (PRL), 200 µl of second AB (sheep plasma containing antibodies against purified rabbit gamma globulin, Pentex Rabbit Gamma Globulin Fraction II) was added to each tube. After a 24 hr incubation at 10C, the precipitate resulting from the addition of second AB was concentrated by centrifugation at 500 x g for 20 min. The supernatant was decanted and the radioactivity contained in the pellet was counted in a Packard Gamma Counter, Model 3375 or 3356. Counting error was maintained at 1% by counting each sample until a total of 10,000 counts was accumulated.

1. Luteinizing Hormone. A five point standard curve prepared in triplicate with porcine LER-786-3 was included in each LH assay. All plasma unknowns were analyzed in duplicates of 300 µl of plasma per tube. On each assay, aliquots of serum from an ovariectomized sow were used as an internal standard and run in triplicate at doses of 300 and 200 µl per tube. Addition of first antibody at a final dilution of 1 to 50,000 precipitated approximately 32% of the radioactive trace.

2. Follicle Stimulating Hormone. In order to conserve a limited supply of the highly purified porcine FSH preparation LER-1132 (66-67), the five point curve which was routinely run on each FSH assay was prepared in triplicate with NIH-PFSH-P1. Although the potency of NIH-PFSH-P1 is only one-twentieth that of LER-1132, the 2 hormone preparations exhibit parallel dose response curves when plotted as log dose vs logit percent counts precipitated (Rayford et al., 1973). Although the plasma hormone concentrations were evaluated against the NIH-PFSH-P1 standard, all unknowns are expressed as ng of LER-1132 (66-67)/ml plasma.

Plasma unknowns were analyzed in duplicates of 500 µl of plasma per

tube. First antibody PR-6-72 was used at a final dilution of 1 to 30,000. An inadequate supply of a suitable FSH serum pool precluded the addition of an internal serum standard to each FSH assay.

3. Prolactin. A suitable prolactin substitute for the highly purified SHE 88.2 reference preparation was also necessary due to the limited supply of the purified hormone. Serum pools from post-partum sows (~24 hrs) were found to contain elevated prolactin concentrations. When this post-partum serum (PPS-1) was plotted as log dose vs logit percent counts precipitated, it was also found to be parallel to the purified SHE 88.2 standard. The pooled post-partum serum was used as the hormone preparation for the standard curves used in this study; however, all unknowns are expressed as ng of SHE 88.2 per ml plasma (500 µl of pooled post-partum serum is equivalent to 12.5 ng of purified porcine PRL SHE 88.2).

Plasma unknowns were run in duplicate at 300 µl plasma per tube. First antibody 3-100-5 was used at a final dilution of 1 to 100,000 with an initial binding ratio of 22%. On each assay, pooled serum was also used as an internal serum standard and run in triplicate at doses of 300, 200 and 100 µl of serum per tube.

4. Analysis of Data. Numerous statistical approaches exist for the calculation of radioimmunoassay data (Rodbard et al., 1969). The method used in this study was designed for use with double-antibody radioimmunoassays (Rodbard and Cooper, 1970). In these assays, the radioactivity in the bound fraction is counted for a fixed period of time and then represented as the $\log_e$ of the percentage of labelled hormone bound to antibody in the absence of unlabelled hormone (logit transformation). The application of "weighted least squares" regression analysis to the logit of percent antibody bound vs the log of the hormone concentration produces a linear

relationship from which dose interpolation can be made.

A Fortran IV computer program was written which uses the log and logit transformations to obtain a linear dose response curve. The initial transformations are followed by unweighted and weighted least squares regression analysis of the standards. Unknown hormone concentrations are estimated from the weighted regression equation and then represented as nanograms of purified hormone per ml of plasma. The computed hormone concentration for each unknown is listed on a computer printout sheet as well as punched on computer cards. The punched output is then used for further statistical computations.

5. Elimination of Between-Assay Variation. The reproducibility of radioimmunoassay data between duplicate samples within a given assay has been shown to be excellent (mean coefficient of variation between duplicates is approximately 3.0%, table 2). However, the large between-assay variation among similar samples included on different assays inflates the error term in a statistical analysis and, consequently, reduces the possibility of finding a significant effect of treatment. In order to decrease this source of error, the data from each assay were adjusted to the grand mean of all the individual internal standards. The adjustment was accomplished by calculating a mean hormone concentration for the internal standard which had been included on each hormone assay. The grand mean of all the internal standards for a given hormone was computed and then compared to the mean hormone concentration for the individual internal standard of a given assay. Each sample within that assay was then adjusted according to the ratio of the internal standards to the grand mean. Using this approach, all the LH and PRL data from 24 different assays were adjusted to the grand mean of their separate internal serum standards. The lack of a

suitable internal FSH standard precluded data adjustment for this hormone.

G. Experimental Design. This experiment was designed as a randomized block experiment, investigating the effects of surgical treatment on plasma hormone concentration (LH, FSH and PRL). The surgical treatments consisted of cannulated intact, sham operated, ovariectomized and hysterectomized pigs in which LH, FSH and PRL concentrations were measured from plasma collected at 6 hr intervals over a period of one estrous cycle. The ovariectomized and hysterectomized pigs were sampled for 20 days (this was the mean estrous cycle length of the 12 control pigs). Each group consisted of 6 animals.

H. Statistical Analysis of Data. The standard statistical procedures which have been used were previously described by Snedecor and Cochran, 1969 and Steel and Torie, 1960. Additional statistical procedures which have been employed are described in this text under sub-heading B of section IV, Results.

IV. RESULTS

A. Validation of Sample Collection Procedure

Since it is not possible to directly quantify the degree of normality between or within animals by any one criterion, the effects of confinement, surgical treatment and continual blood removal on the physiological state of the experimental animals were accessed by evaluating 5 independent parameters.

1. General Physical Appearance and Behavior of Animals While in Confinement. The greatest degree of variability in the general physical appearance of the experimental animals occurred during the first 6 days of cannulation. The lathargic state and reduced food and water intake noted in some animals during the first week of cannulation were most likely the direct result of inherent variability in the animal's ability to recover from surgical trauma. By the third day after surgery, the majority of the animals were eating their daily ration of feed and were observed standing in their stalls at some time during sample collection. The physical activity of the hysterectomized animals was suppressed during the first week of blood collection; however, the food consumption in this group of animals was not different from the other surgical treatments. Food consumption during the recovery period following surgery was independent of surgical treatment. The appetite of the animals appeared to be related to their ability to recover from the stress of surgery. By the end of the first week of blood collection, all animals had adapted to their new surroundings and their activity and food consumption remained comparable to that of "normal", untreated animals.

After full recovery, the animals remained alert and responsive during

the remainder of their confinement in the stalls (~25 days). They became extremely alert to any sounds associated with the arrival of food and routinely greeted the farm manager with robust vocalizations. The overall excitement generated during feeding time was sufficient to increase packed cell volumes by 5 to 10%. In order to reduce the effect of feeding on blood collection, the animals were routinely fed at 1500 hrs.

After the animals had adapted to the collection schedule, frequent visits by the investigator were routinely ignored or on occasion acknowledged with a sedate grunt. This general lack of concern allowed blood samples to be removed from sleeping animals during the 2400 and 0600 hr collection periods without interrupting their sleep.

2. Display of Heat. The degree of activity associated with insuing behavioral heat varies from animal to animal. Detection of the onset of behavioral heat in confined animals was found to be difficult in some cases. In some instances, the extremely excitable state of the sows at the onset of estrus permitted a more accurate estimate of the onset of behavioral heat. Two of the 12 control animals originally assigned to this study did not show any noticeable signs of behavioral heat during cannulation. Any control or sham operated animal which failed to display a detectable heat was replaced.

3. Packed Cell Data. The physical appearance, behavior, appetite and display of behavioral heat are parameters which would most likely be affected when an animal is exposed to a noxious environment. However, subtle changes in the blood physiology of experimental animals, resulting from the continuous loss of blood, could not be accessed by gross physical appearance. In order to evaluate the effect of continual blood loss, hematocrits were recorded after the removal of each blood sample.

The packed cell data from day 1 until day 22 of sample collection were regressed with time to determine if the slope of the regression line was significantly different from zero (table 3). A significant, negative slope would indicate that the number of red blood cells being removed during sample collection was greater than the number of cells being formed. Regression analysis of the packed cell data over the 22-day collection period indicated that 2 animals had a negative regression line which was significantly different ($P<.05$) from zero. Analysis of the packed cell data from these animals indicated that the reduction in packed cell volume was less than three tenths of one percent per day. The mean hematocrit values for these 2 animals on the twenty-second day of cannulation falls within the 95% confidence interval about the grand mean for all the daily hematocrits at the twenty-second day of sample collection. Since the percentage of packed cells does not fall outside of the confidence interval of the sample population, it is unlikely that the depletion of packed cells during the first 22 days of sample collection had a deleterious effect on the physiology of these animals.

The daily mean packed cell volume at days 1, 12 and 22 (tables 4 and 5) of sample collection was analyzed with a randomized complete block analysis of variance design. An analysis of the packed cell data on the first day of sample collection indicated a significant effect ($P<.01$) of treatment. An analysis of the treatment means by the Student Newman-Keuls' test (SNK Test) indicated that the percentage of packed cells in the hysterectomized animals was significantly lower ($P<.05$) than the hematocrit ratios for the other 3 treatments. An analysis of variance of the hematocrit data on the twelfth day of sample collection still revealed a significant effect of treatment at the 5 percent level of significance. The packed cell volumes of the sham and hysterectomized groups were found to be significantly

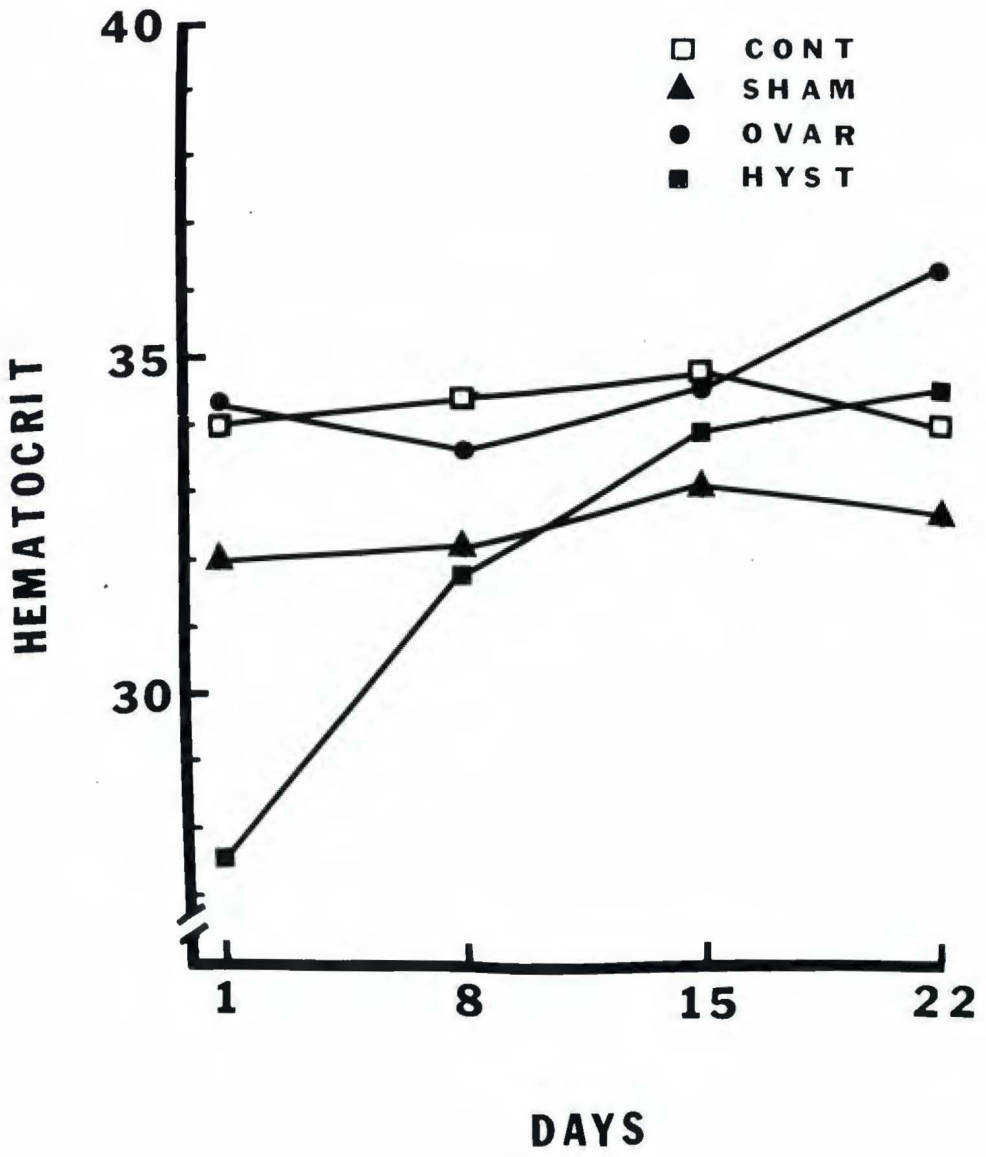
less ($P<.05$) than the hematocrit values for the ovariectomized and control groups. At the twenty-second day of sample collection, surgical treatment no longer had a significant effect ($P>.05$) on the percentage of packed cells in the blood.

An initial interpretation of the packed cell data indicates that the hematocrit values for the hysterectomized animals were significantly less ($P<.05$) than the hematocrits of the other 3 treatments at the beginning of the sample collection period. During the first 12 days of sample collection, the packed cell volumes in the hysterectomized animals increased so that they were no longer significantly different ($P>.05$) from the sham treated animals.

A factorial analysis of variance was used to obtain additional information regarding the effect of time of sample collection and surgical treatment on packed cell volume (table 6). An analysis of the data indicated that a significant interaction existed ($P<.05$) between surgical treatment and time. With a significant interaction, the factors are not independent of one another. The simple effects of a factor may differ; however, the magnitude of any simple effect depends upon the level of the other factor of the interaction term (figure 2). The data obtained from this analysis must be interpreted cautiously, due to the lack of independence in the packed cell data with regard to time; however, the fact that a significant interaction exists is noteworthy and should merit further consideration. The treatment means were not found to be significantly different ($P>.05$) when analyzed by SNK test.

The depressed hematocrits in the hysterectomized animals, during the first week of sample collection, may be due to blood loss associated with the removal of the uterus or the extended recovery period required for this treatment. The hysterectomized animals were not as active as their coun-

Figure 2. The effect of day of blood collection on the percent of packed cells in the control, sham, ovariectomized and hysterectomized animals.


40
35
30
HEMATOCRIT
CONT
SHAM
OVAR
HYST
1
8
15
22
DAYS

terparts during the first week of sample collection. The lack of movement and decreased muscle tone observed in these animals, during the first week of sample collection, might indirectly reduce packed cell volume. This hypothesis is tentatively supported by the fact that the hematocrit values during the midnight collection period are significantly less ($P<.05$) than the packed cell volumes during the 0600, 1200 and 1800 hr collection periods (table 7). Thus, it appears that the lack of muscular movement in conjunction with a drop in blood pressure allows the cellular elements within the blood to settle-out, decreasing the percentage of circulating packed cells. Similar fluctuations in hematocrit values have been observed in humans and have been attributed to a redistribution of body fluids resulting from posture and physical activity (Mills, 1966). The fact that the hematocrits in the hysterectomized animals rise during the first week of sample collection and are not significantly different ($P>.05$) from the sham treated animals during the remainder of the collection period, further supports the hypothesis that the net loss of blood cells during sample collection does not exceed the net production of blood cells during this period.

4. Differential Cell Counts. In order to evaluate the quantitative relationship between red and white blood cells during sample collection, cell counts were made during the middle and end of the 22-day collection period. No significant differences ($P>.05$) in red or white cells were noted within surgical treatment or during the time of sample collection (table 8). The number of red (3.8 to 10.0 x $10^6/mm^3$) and white (1.9 to 2.6 x $10^4/mm^3$) blood cells was found to be similar to the values which have been previously reported in the literature (Calhoun and Smith, 1970).

5. Post-Experimental Carcass Examination. Upon conclusion of the experiments, the animals were sacrificed and the reproductive organs were removed and examined. All the carcasses passed federal meat inspection.

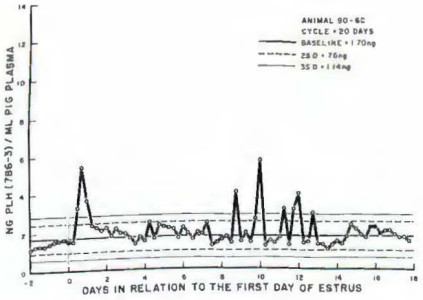
The reproductive tracts in the control and sham operated animals were found to be normal. The uteri in the ovariectomized group were infantile in appearance, but otherwise normal. A precise count of follicles and CL was not possible due to the presence of post-operative adhesions.

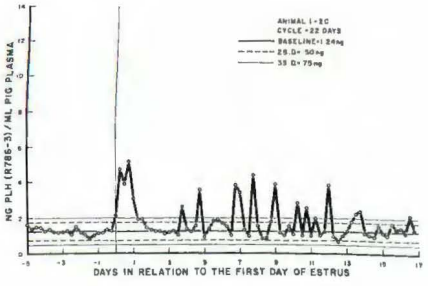
The mild temperment, sedate behavior and the large size of the domestic swine make this animal a perfect choice for long-term cannulation studies. The animals adapted very readily to confinement and remained healthy during sample collection. In addition, they were capable of tolerating a daily loss of 40 ml of blood for as long as 1 month without any substantial change in packed cell volume or red and white blood cell numbers.

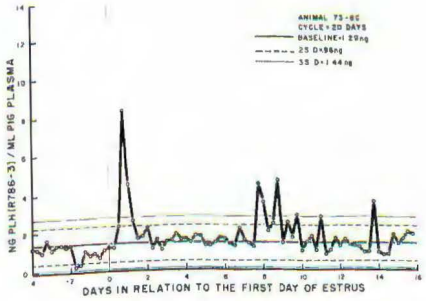
B. Development of Techniques For Quantitative Expression of Plasma Hormone Concentration Changes

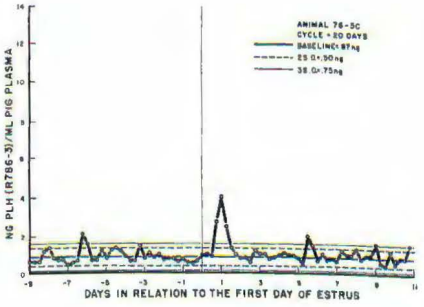
An increase in the availability of precise, sensitive and accurate radioimmunoassays has allowed investigators to quantify gonadotropins in the peripheral plasma of many different species. Unfortunately, the analysis of plasma hormone fluctuations has been limited to little more than the ordinary "variate-time" graph where the hormone concentrations are plotted on the Y-axis against time on the X-axis, thus depicting hormone concentration as a function of time (figure 3). Analysis of the variate-time graph shows that hormone fluctuations occur around a certain central value which represents the general basal plasma concentration. This central or basal value may be defined as the concentration from which all hormone fluctuations (peaks) deviate. Though its meaning is clear and generally accepted, its calculation and subsequently, its expression as a statistic has been hindered by the great variation in the data derived from biological studies. When the concentrations remain relatively constant and approximate a straight line, the basal value may be represented

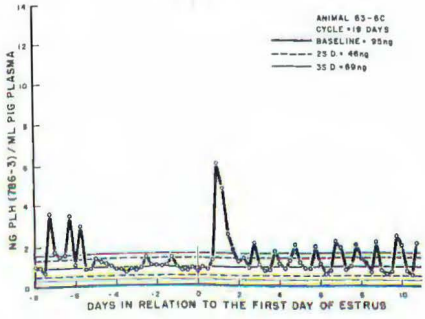
Figure 3. Temporal pattern of plasma LH concentrations during the estrous cycle of 6 control swine. All data collections begin at 0600 hrs on the third day of cannulation.

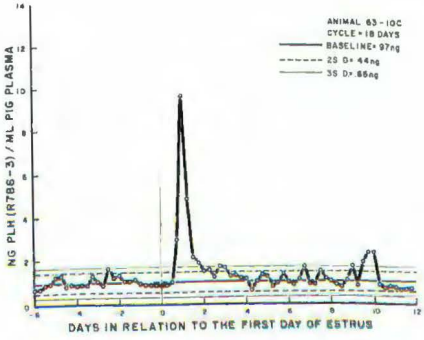

ANIMAL 90-6C
CYCLE = 20 DAYS
BASELINE = 170ng
25 D = 76ng
35 D = 114ng
NG PLH (786-3) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 1-2C
CYCLE = 22 DAYS
BASELINE = 124ng
25 D = 50ng
35 D = 75ng
NG PLH (R786-3) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 73-6C
CYCLE = 20 DAYS
BASELINE = 129ng
25 D = 96ng
35 D = 144ng
NG PLH (R786-3) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 76-5C
CYCLE = 20 DAYS
BASELINE = 97ng
25 D = 50ng
35 D = 75ng
NG PLH (R786-3) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 63-6C
CYCLE = 18 DAYS
BASELINE = 95ng
25 D = 66ng
35 D = 89ng
NG PLH (786-3) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 63-10C
CYCLE = 18 DAYS
BASELINE = 97ng
25 D = 44ng
35 D = 66ng
NG PLH (R786-3) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS

by an average of these concentrations over the entire estrous cycle (arithmetic, harmonic or geometric mean, median, mode, middle value). The fluctuation about this basal value could be expressed as a variance term (e. g., mean$\pm$1 standard deviation).

In order to evaluate all hormone fluctuations, a method must be established for objectively determining the mean and variance of those hormone concentrations within an estrous cycle which constitute a "basal population" of concentrations and just as objectively separate the "basal population" and its mean, hereafter designated "baseline", from abrupt deviations from the baseline ("peaks") of varying duration and concentration (e. g., the preovulatory rise in LH concentration before ovulation). A sound mathematical estimate of baseline and its variance is an essential prerequisite for the evaluation of hormone concentrations which deviate from the basal hormone concentration. In an attempt to reduce some of the subjectivity associated with the derivation of baseline, a simple statistical procedure has been developed and used to obtain estimates of baseline and its variance. This procedure is based on the following assumptions:

1. Since hormone concentrations (e. g., LH) can be detected at any time during the estrous cycle, acute physiological changes (e. g., ovulation) in the target tissue resulting from the presence of a specific hormone must be due to a change in hormone concentration.
2. If the induction of an acute physiological change in a target tissue is the result of a change in hormone concentration, then the control exerted by the hormone must be at least biphasic in nature (the hormone circulates at concentrations which effect no change other than the possible maintenance of cellular integrity of the target tissue, passive stimulation, or circulates at concentra-

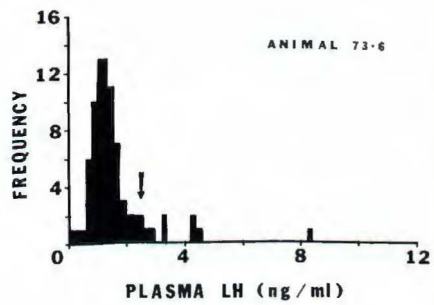
tions which are capable of inducing morphological and or chemical changes in the target tissue, active stimulation).

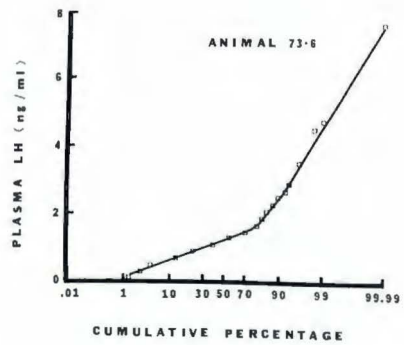
If the induction of an acute physiological change in a target tissue is the result of a significant change in hormone concentration, at least 2 populations of hormone concentrations must exist, 1 population associated with passive stimulation and the other population with active stimulation. In order to examine this possibility, LH, FSH and PRL concentrations collected during 1 estrous cycle from animal 73-6C were depicted as frequency distributions (figure 4). An initial inspection of the data shows that all 3 distributions display positive skewness (LH and PRL, $P < .01$; FSH, $P < .05$).

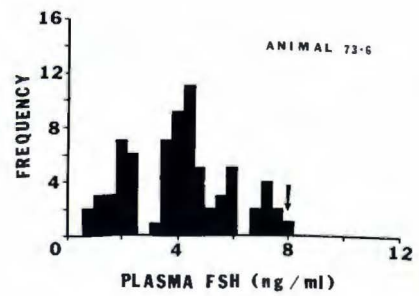
When hormone concentrations are depicted in a variate-time graph (figure 3), it can be seen that the distribution of hormone concentrations is a function of the formula which depicts the path of the variable with respect to time. Changes in hormone concentration are roughly proportional to changes in the velocity of the variable. Sollberger (1965) has suggested that equidistant sampling from 1 variate without preservation of the time location will lead to a skewed distribution if the velocity of the variable is not fixed. Since the hormone concentrations in this study have been collected at equidistant intervals, it can be assumed that the significantly skewed appearance of the hormone frequency distributions are the result of a change in the velocity of the variable.

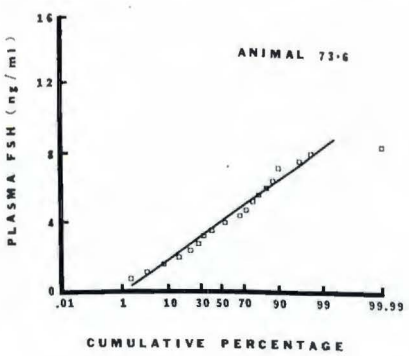
The hormone concentrations making up the variate-time graph appear to be made up of 2 distinct populations, 1 of which consists of a large number of concentrations with a small magnitude (e. g., those concentrations associated with minor changes in the velocity of the variate, passive stimulation) and the other consisting of a smaller number of concentrations with a large magnitude (e. g., concentrations associated with a major change in

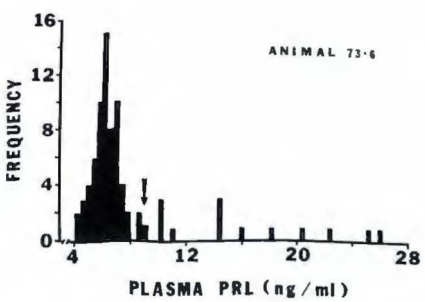
Figure 4. The frequency distributions and cumulative probability plots of porcine LH, FSH and PRL plasma concentrations during the estrous cycle of animal 73-6C.

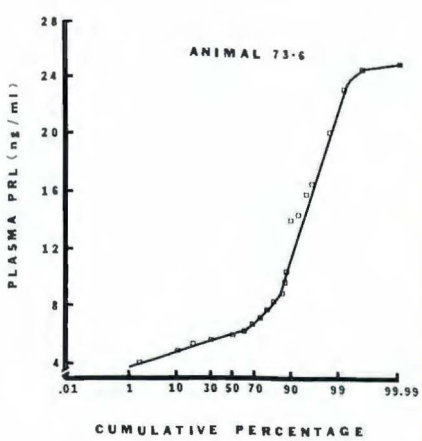

ANIMAL 73-6
FREQUENCY
16
12
8
4
0
4
8
12
PLASMA LH (ng/ml)


ANIMAL 73-6
PLASMA LH (ng/ml)
8
6
4
2
0
.01
1
10
30 50 70
90
99
99.99
CUMULATIVE PERCENTAGE


ANIMAL 73-6
FREQUENCY
16
12
8
4
0
4
8
12
PLASMA FSH (ng/ml)


ANIMAL 73-6
PLASMA FSH (ng/ml)
16
12
8
4
0
.01
1
10
30 50 70
90
99
99.99
CUMULATIVE PERCENTAGE


ANIMAL 73-6
FREQUENCY
16
12
8
4
0
4
12
20
28
PLASMA PRL (ng/ml)


ANIMAL 73-6
PLASMA PRL (ng/ml)
28
24
20
16
12
8
4
.01
1
10
30 50 70
90
99
99.99
CUMULATIVE PERCENTAGE

the velocity of the variate, active stimulation). In order to determine if estrous cycle hormone concentrations exist as multiple distributions, the LH, FSH and PRL data from animal 73-6C were expressed as cumulative percentages and plotted on probability graph paper according to Harding (1949). Cumulative probability paper is arranged so that when any normally distributed population is plotted, the points all fall on a straight line. If a bimodal or polymodal distribution is composed of distributions which are themselves normally distributed, it will be depicted as a curve (e. g., the resultant of 2 or more straight lines) when plotted on probability paper (Harding, 1949). It can be seen in figure 4 that the LH, FSH and PRL hormone concentrations collected during 1 estrous cycle consist of at least 2 distributions.

Since hormone concentrations are restricted to minor fluctuations around baseline during the major portion of the estrous cycle, one does not obtain a large number of observations from the second population of hormone concentrations associated with active stimulation and the second population cannot be defined as precisely and accurately as the basal population. The frequency distribution plot of hormone concentrations clearly showed that a large number of hormone concentrations were associated with the basal population.

The demarcation point of each basal population was estimated by testing for the presence of positive skewness. In order to obtain an objective estimate of the mean and variance of the basal population, the hormone concentrations from 1 estrous cycle were ranked in ascending order and the degree of skewness (g_1, statistical symbol for the measure of skewness) was estimated. The presence of 2 populations of concentrations was indicated by a significant positive skewness estimate (tables 9 to 11). As concentrations to the right of the basal population were excluded from

the estimate of skewness, the numerical estimate of g_1 approached zero (the value of g_1 for a normally distributed population is zero). Concentrations were removed until the estimate of skewness was no longer significant ($P>.05$) indicating that the remaining concentrations constituted a normally distributed population (basal population). The mean and variance of the basal population were calculated from the remaining hormone concentrations.

The truncation procedure described above was used to estimate LH, FSH and PRL baselines and standard deviations (table 12). In all of the treatments, hormone concentrations which fell outside of 2 standard deviations from the baseline were considered to be significantly different from baseline. Hormone concentrations which were significantly greater than baseline were considered to be significant "peaks" (peak: 1 or more consecutive hormone concentrations whose directional slope changes from positive to negative with respect to baseline).

In order to compare the data from this study with hormone concentrations which have been previously reported for the pig, peak magnitudes were expressed in terms of absolute increase in hormone concentration (e. g., ng/ml of hormone measured from 0). Hormone concentrations associated with significant peaks were also expressed in terms of ng of hormone/ml plasma - baseline (BL) and ng of hormone/ml plasma - (baseline + 2 standard deviations, BL + 2 SD). Peak durations were computed from baseline. In those cases where hormone concentrations did not return to sub-baseline levels, durations were estimated by extrapolating the estimated rate of change to baseline. Peak intervals were computed by measuring the distance between parallel lines drawn perpendicular to baseline from points located in the center of each peak at 1/2 peak height.

C. Analysis of Plasma LH Concentrations Throughout the Porcine Estrous Cycle

1. Introduction. A great deal of time has been spent in attempting to determine the luteotropic complex in the major domestic species. Exogenous estrogens and LH when given during the luteal phase of the estrous cycle have both been shown to increase progesterone secretion in the cow, sheep and pig; however, the lack of biological data relating to the endogenous concentrations of these hormones during the estrous cycle has prevented investigators from demonstrating that these hormones function in a luteotropic manner in the intact animals.

The injection of exogenous LH during the luteal phase of the estrous cycle has been shown to prolong the life span of the corpus luteum in the cow (Donaldson and Hansel, 1965). The observation of an endogenous increase in circulating LH concentration during the luteal phase of the bovine estrous cycle (Schams and Karg, 1969; Henricks et al., 1970; Snook et al., 1971) further supports the hypothesis that LH is luteotropic in this species. In addition to showing that LH concentrations were elevated during the luteal phase of the estrous cycle, Snook et al. (1971) has also shown endogenous LH and progesterone concentrations to be significantly correlated during the luteal phase of the cycle (days 3 to 15).

The injection (Anderson et al., 1967), infusion (Cook et al., 1969) or removal of LH with anti-LH antibodies (Spies et al., 1967) have all been shown to alter progesterone concentration in the pig. Although it has been hypothesized that the porcine corpus luteum can function independently of hypophyseal support (Sammelwitz et al., 1961; Brinkley et al., 1964 a,b; du Mesnil du Buisson and Leglise, 1963), these data support the hypothesis that LH may be luteotropic during the estrous cycle of the pig. The following study was initiated in order to more critically eval-

uate the importance of endogenous LH during the porcine estrous cycle.

2. Materials and Methods. Indwelling cannulae were inserted into the vena cava of 12 nulliparous Yorkshire sows after they had completed at least 1 estrous cycle (18 to 22 days). Six of the 12 sows, hereafter designated as sham treated, were also laparotomized for inspection of the reproductive tract. The 12 animals were housed in special holding stalls at the Large Animal Laboratory at the University of Maryland. Blood samples were collected at 6 hr intervals beginning at 0600 hrs and continuing for the duration of the estrous cycle; however, samples collected during the first 3 days after surgery were not included in the data analysis. The plasma was removed by centrifugation and stored frozen until assayed by radioimmunoassay (Rayford et al., 1971).

The significance of all hormone fluctuations was objectively evaluated in relation to the mean of the basal hormone population (sub-heading B of section IV., Results).

3. Results. A representative example of the temporal pattern of plasma LH concentrations in the control animals are shown in figure 3. The mean baseline estimate for the 6 control animals during their 19.83± 1.33 (1 standard deviation, 1 SD) day estrous cycle was 1.17±.31 ng LH/ml plasma (table 13). Other procedures were also used to obtain estimates of baseline (grand mean, median and mode) in order to compare the truncation procedure to other techniques which have been previously reported in the literature (table 13).

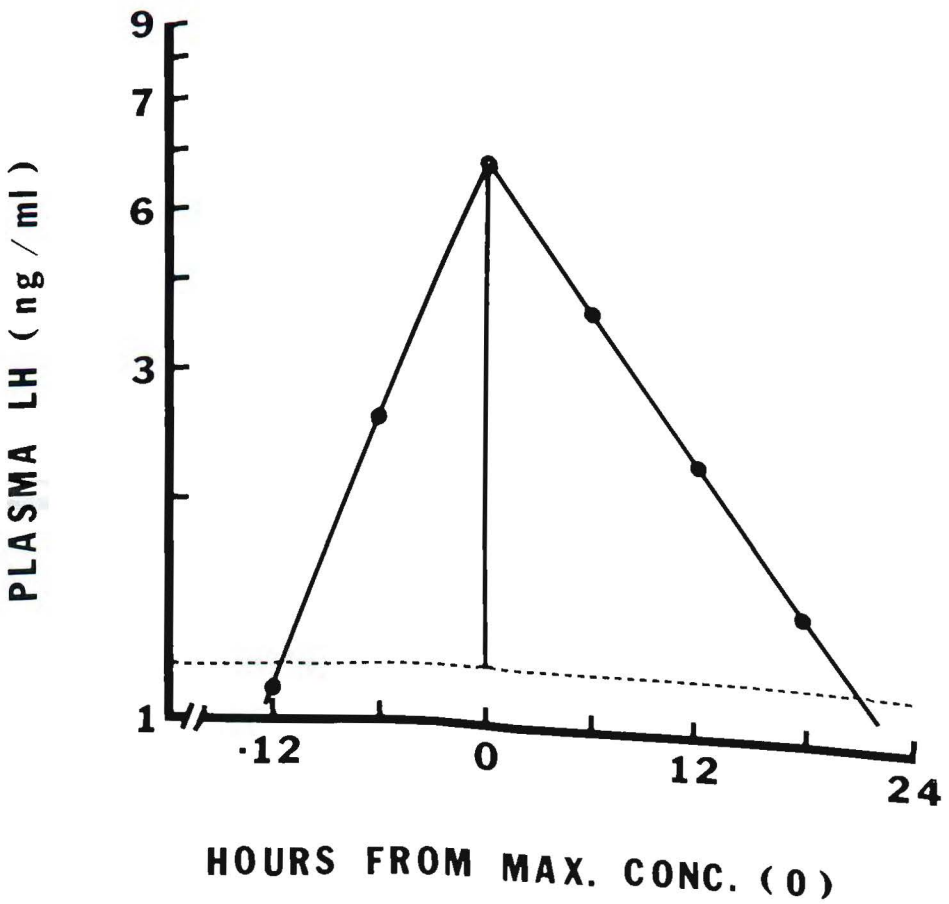
A preovulatory LH (PrOvLH) concentration of 6.41±2.12 ng LH/ml plasma routinely peaked at either 2400 hrs on day 0 or 0600 hrs on day 1 (table 14). The concentration of LH in the preovulatory peak was 5.44 times greater than baseline and 3.63 times greater than baseline plus 2 standard deviations above baseline (BL + 2 SD). In addition to expressing LH

concentrations in terms of absolute ng of LH/ml plasma, hormone concentrations were also expressed in terms of ng of LH/ml plasma minus baseline and ng/ml plasma minus (BL + 2 SD). Preovulatory plasma LH concentrations of 5.24±2.16 ng (ng of LH - BL) remained elevated for 28.52± 6.11 hrs before returning to baseline. The duration of the preovulatory peak estimated from baseline was similar to the duration estimate of 29.3± 10.59 hrs which represents the length of time that the LH concentrations exceeded BL + 2 SD. The greater variance in the BL + 2 SD estimate was due to the "shoulder" on the descending side of the peak (figure 3).

The LH concentration in the ascending or descending sides of the preovulatory peak could be depicted as a straight line when the $\log_{10}$ of the LH concentration was plotted as a function of time (figure 5). Least squares regression analysis of the LH concentrations in the ascending and descending halves of the preovulatory peak indicated that a significant percentage of the variation in the total sum of squares could be attributed to regression (correlation coefficient of ascending slope r=.944** and descending slope r= -.892** for 15 and 21 df). The formula for the regression equation on the ascending and descending halves of the preovulatory peak was Y $\log_{10}$=.775+.060X and Y $\log_{10}$=.764-.032X, respectively. The regression coefficients were significantly different from 0 (ascending t=11.05**, df=15 and descending t= -9.05**, df=21 for B=0). The slopes of the ascending and descending halves of the preovulatory peak were also significantly different (t=2.37*, df=36).

The time required for the LH concentration to rise from baseline to maximum plasma concentration during the preovulatory release was 11.62 hrs (8.6 hrs from BL + 2 SD to peak) while an estimated 21.56 hrs (15.99 hrs from peak to BL + 2 SD) was required for LH concentrations to return to baseline (estimates made with the least squares regression equation).

Figure 5. Least squares regression analysis of the slope of the ascending and descending halves of the preovulatory LH peak. The slope of the ascending half of the preovulatory peak (Y $\log_{10}$=.775+.060X, r=9.44**) was significantly different (t=2.37*, df=36) from the slope of the descending half of the peak (Y $\log_{10}$=.764-.032X, r= -.892**). Plasma LH reached maximum concentration in 11.62 hrs and returned to baseline (----) 21.56 hrs after peak concentrations were reached.


9
7
6
3
1
PLASMA LH (ng / ml)
·12
0
12
24
HOURS FROM MAX. CONC. (0)

The graphical estimates of the time required for preovulatory LH concentrations to reach maximum concentrations and return to baseline were 14.01 ±6.28 and 44.1±18.6 hrs.

In addition to the preovulatory LH peak, an average of 8.5±3.54 (range 5 to 14) luteal phase peaks was observed during each estrous cycle (at least 1 concentration in each of these peaks was significantly greater than baseline). Approximately 68.6% of the luteal phase peaks (5.83± 2.56 peaks/estrous cycle) contain only 1 concentration significantly different from baseline (class 1 peaks). These peaks reached maximum concentrations of 2.34±.75 ng LH/ml plasma and declined to baseline over a period of 11.78±1.76 hrs during days 2 to 17 (duration estimated by extrapolation to baseline). The remaining 31.4% of the luteal phase peaks (2.67±1.03 peaks/estrous cycle) contained 2 or more concentrations which were significantly different from baseline (class 2 peaks) and reached concentrations of 3.21±1.20 ng LH/ml plasma between days 2 to 13 (table 14). This second group of luteal phase peaks remained elevated 18.30± 4.22 hrs before returning to baseline concentrations. This was approximately two-thirds as long as the duration estimate of the preovulatory LH peak. The greatest increase in detectable hormone among the mid-cycle peaks occurred between days 7 to 13 and reached a mean concentration of 3.79±1.39 ng LH/ml plasma (3.2 fold increase above baseline). The average interval between luteal phase peaks was 37.48±13.31 hrs (1.56±.55 days, table 15).

The time interval between the preovulatory LH (PrOvLH) peak and the first mid-cycle LH (FMCLH) peak was 3.68±2.07 days. The last mid-cycle LH (LMCLH) peak occurred 14.78±1.89 days after the PrOvLH peak. The time interval between the PrOvLH peak and the average mid-cycle LH ($\bar{x}$MCLH) peak was 9.04±.78 days. The time interval between the FMCLH peak and the

LMCLH peak was 11.01±2.43 days. The length of time from the LMCLH peak to the PrOvLH peak was 5.24±2.22 days (table 15). LH concentrations usually remained below baseline during this interval.

Approximately 16.5±5.68 LH concentrations (concentrations/estrous cycle) were significantly different from baseline (table 13). Sixty-six percent of these concentrations were observed to occur during the 2400 or 0600 hr collection periods (χ^2=17.72**, df=3). The appearance of elevated plasma LH concentrations during the night hrs was also reflected in the means of the 4 collection periods (figure 6). A randomized block analysis of variance of LH concentrations collected during the 4 collection periods (table 16) indicated a significant effect of both treatment (time of day) and block (animal). Student Newman-Keuls' test for comparison of treatment means indicated that the means of the 2400 and 0600 hr collection periods were significantly greater ($P<.01$) than the means at 1200 and 1800 hrs; however, no significant differences existed between the means of the 0600 and 2400 and the 1200 and 1800 hr collection periods. The significant block effect was due to animal variation in both the number and magnitude of the mid-cycle peaks.

The use of statistics in the analysis of hormone fluctuations during the estrous cycle serves as a basis for objectively determining which hormone concentrations are statistically different from baseline. Although it is not possible to determine the biological significance of individual concentrations, a graphical and statistical analysis of the temporal pattern of the significant concentrations within the estrous cycle may offer a means of evaluating their relative biological importance. A graph of all the plasma LH concentrations which were significantly greater than baseline is presented in figure 7.

A randomized block analysis of variance of those hormone concentra-

Figure 6. The mean and standard deviation of plasma LH concentrations at the 0600, 1200, 1800 and 2400 hr collection periods during the porcine estrous cycle. The mean plasma LH concentrations at the 2400 and 0600 hr collection periods were significantly greater ($P<.01$) than the LH concentrations at the 1200 and 1800 hr collection periods.

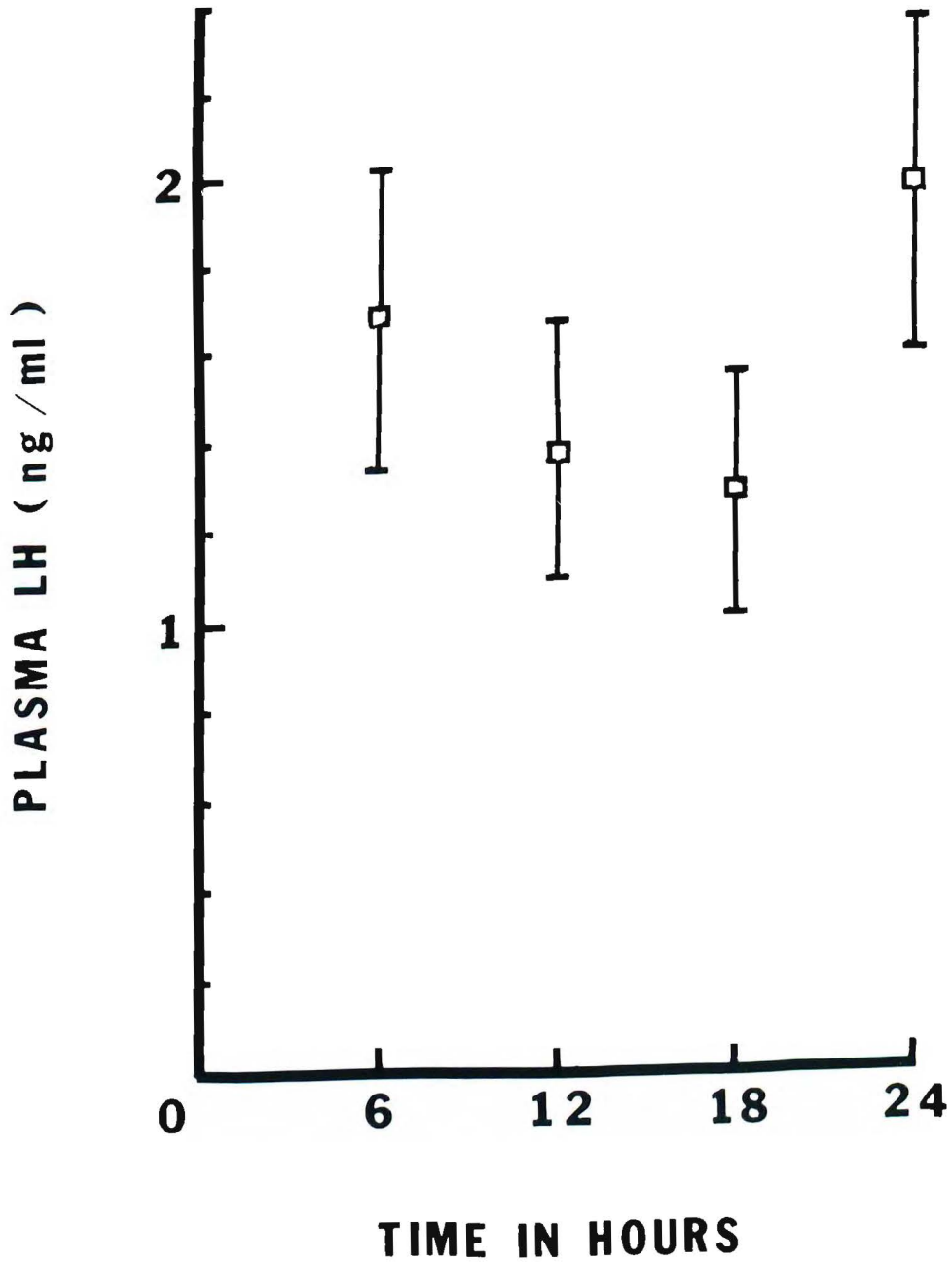
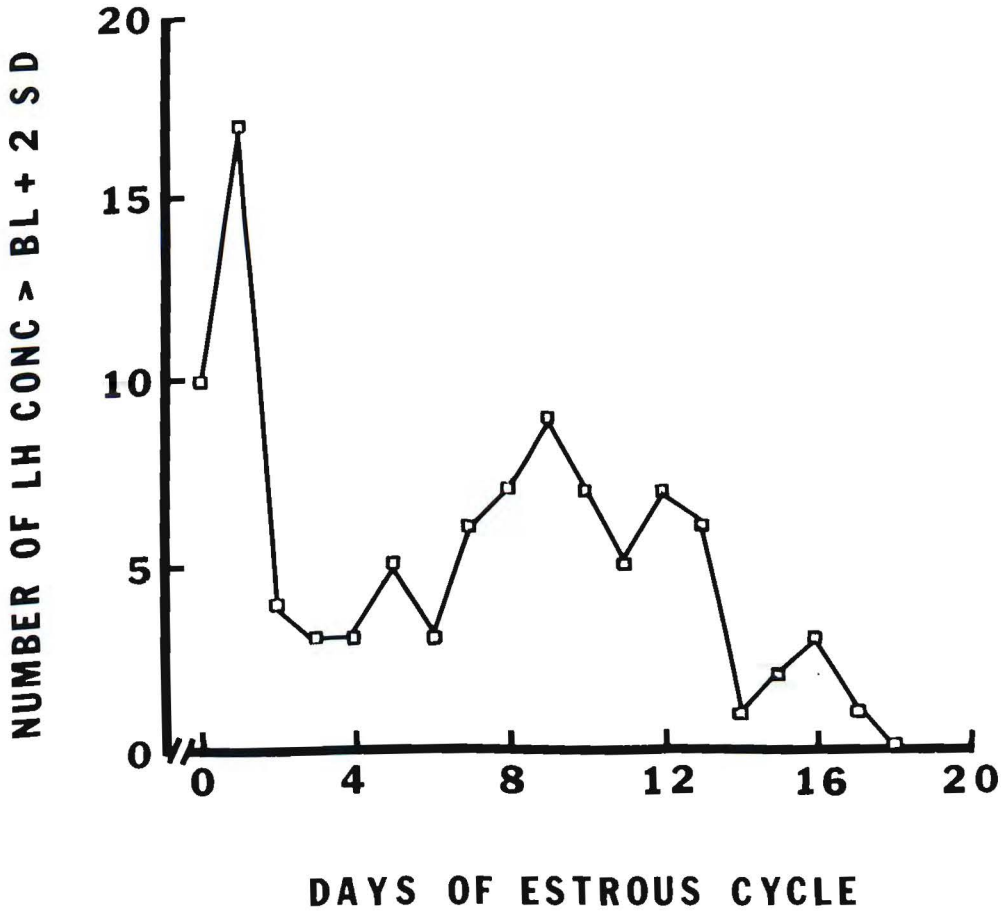

PLASMA LH (ng / ml)
2
1
0
6
12
18
24
TIME IN HOURS

Figure 7. The distribution of plasma LH concentrations which were significantly greater than baseline, in relation to the day of the estrous cycle.


NUMBER OF LH CONC > BL + 2 SD
20
15
10
5
0
0
4
8
12
16
20
DAYS OF ESTROUS CYCLE

tions which were significantly greater than baseline indicated a significant effect of interval ($P<.01$). The number of concentrations found to be significantly greater than baseline during days -1 to 2 was not significantly different ($P>.05$) from the number of concentrations excluded during days 7 to 10, and 11 to 14, but was significantly greater than those excluded during day 3 to 6 and 15 to -2 (table 17). Since the number of concentrations found to be significantly greater than baseline during the preovulatory release (days -1 to 2) was not significantly different from the number of concentrations excluded during days 7 to 10 and 11 to 12, LH release during days 7 to 12 may also have biological significance.

The analysis of sample means for the control and sham operated animals revealed no significant differences at the 5% level of significance (t-test for comparison of 2 sample means, tables 14 and 15). Since no significant differences were found between the means of the control and sham treated animals, further statistical analyses were based on the grand mean for these 2 treatments.

The concentration of LH in the preovulatory peak (pooled data from control and sham operated animals) was significantly greater than the concentration of LH in either class 1 (PrOvLH 7.06±2.40 ng/ml vs class 1 2.29±.55 ng/ml, t=6.70**, df=22) or class 2 luteal phase peaks (PrOvLH 7.06±2.40 ng/ml vs class 2 2.86±1.04 ng/ml, t=5.12**, df=20, table 14). The length of time that the LH concentration remained above baseline was significantly greater in the preovulatory LH peak than in either class 1 (PrOvLH 28.75±4.76 hrs vs class 1 12.65±1.79 hrs, t=10.97**, df=22) or class 2 luteal phase peaks (PrOvLH 28.75±4.76 hrs vs class 2 18.61±3.49 hrs, t=5.59**, df=20). The concentration of LH in class 1 and class 2 luteal phase peaks was not significantly different ($P>.05$) (class 1 2.29±

.55 ng/ml vs class 2 2.86±1.04 ng/ml, t=1.66, df=20); however, class 1 peaks returned to baseline more rapidly than class 2 peaks (class 1 12.65 ±1.79 hrs vs class 2 18.61±3.49 hrs, t=5.20**, df=20).

4. Discussion. The truncation procedure used to estimate the mean and variance of a basal population of hormone concentrations has proven to be an efficient and objective means of evaluating hormone fluctuations. Establishing a statistic which is representative of the mean of the basal hormone population serves as a basis from which all other deviations can be objectively evaluated regardless of their concentrations or time of occurrence within the estrous cycle.

The analysis of plasma during the porcine estrous cycle revealed a diurnal variation in LH concentrations, with LH concentrations high at 2400 to 0600 and low at 1200 to 1800 hrs. In 11 of the 12 control and sham treated swine, preovulatory LH concentrations reached maximum concentrations at 2400 on day 0 or 0600 hrs on day 1. Seventy-three percent of the LH concentrations associated with significant peaks also reached maximum concentrations during the 2400 or 0600 hr collection periods.

Diurnal variations in plasma LH concentrations have been shown to occur in the cow (Madan and Johnson, 1971) and rat (Gay et al., 1970; Lawton and Smith, 1970). Human LH and FSH concentrations also peak during the night hrs (Rubin et al., 1972; Kapen et al., 1973). Rubin et al. (1972) found LH concentrations to be higher during periods of sleep (rapid-eye-movement, REM) in normal men while in the rat Clemens et al. (1972) found a positive correlation between REM periods and LH release. The occurrence of elevations in plasma LH during the night hrs may be due to changing concentrations of central nervous system neurotransmitters since alterations in catecholamine concentrations are thought to control both REM sleep (Jouvet, 1969) and the secretion of LH (Wurtman, 1971).

LH concentrations associated with the preovulatory surge were found to be 2 to 3 times greater than those which have been reported in the literature; however, LH concentrations during the remainder of the estrous cycle were in good agreement with previously reported levels (Niswender et al., 1970; Tillson et al., 1970; Rayford et al., 1971; Henricks et al., 1972). The elevated hormone concentrations associated with the preovulatory LH surge were presumably due to an increase in sampling frequency (6 hr blood collections) as well as a reduction in stress associated with blood removal (blood samples were routinely withdrawn from nonrestrained, conscious or sleeping swine without physical or emotional disturbance to the animals).

Significant increases in plasma LH concentrations were observed throughout the luteal phase of the porcine estrous cycle (8.75±2.16 peaks/estrous cycle). The largest mid-cycle peaks occurred between days 7 to 13 (mean 10.5±2.78 days) and reached maximum concentrations of 3.56±1.05 ng LH/ml plasma. The concentration of LH associated with the largest mid-cycle LH peaks was 3 times greater than baseline and approximately one-half as great as the preovulatory LH surge. These luteal phase peaks occur at a time when plasma progesterone concentrations are known to be rising (Edqvist and Lamm, 1971; Henricks et al., 1972). The infusion of LH into the pig has been shown to significantly increase progesterone secretion on day 10 (Cook et al., 1969), while the removal of LH between days 7 to 11 with anti-LH antibodies causes a 42% reduction in luteal progesterone concentration (Spies et al., 1967). Similar, but less frequent, luteal phase peaks have been observed in the cow (Schams and Karg, 1969; Henricks et al., 1970; Snook et al., 1971) and sheep (Pelletier et al., 1968; Wheatley and Radford, 1969; Scaramuzzi et al., 1970). Snook et al. (1971) found a significant positive correlation between serum

progesterone and LH concentrations during the luteal phase of the bovine estrous cycle. Although data do exist to support the hypothesis that the mid-cycle LH peaks may be luteotropic in the pig, LH is not essential to the structural maintenance of the corpus luteum in the non-pregnant sow (Brinkley et al., 1964 a,b; du Mesnil du Buisson and Leglise, 1963).

In addition to the hypothesized luteotropic effect of the mid-cycle LH peaks, LH fluctuations during the middle and latter part of the porcine estrous cycle parallel changes in the growth and atresia of anovulatory follicles (large follicles which develop during the estrous cycle but are not ovulated during estrus, Green, 1950). The largest anovulatory follicles (7 to 8 mm, Green, 1950; Parlow et al., 1964) occur at a time when mid-cycle plasma estrogen (Henricks et al., 1972) and LH reaches maximum concentrations. Karg et al. (1967) and Snook et al. (1971) have suggested that rising serum LH concentrations during the luteal phase of the bovine estrous cycle may be associated with the growth and atresia of anovulatory follicles.

The lowest plasma LH concentrations were observed during the follicular phase of the porcine estrous cycle (figure 3). Sub-baseline LH concentrations were routinely observed for several days prior to the preovulatory LH surge. The suppression of LH during the follicular phase of the estrous cycle may be due to an initial hypothalamic inhibitory effect of estrogen (Swerdloff et al., 1970) and the removal of circulating LH by the growing follicles. The apparent inability of progestogens to suppress LH secretion during the luteal phase of the estrous cycle is distinctly different from the constant inhibitory effect observed during the follicular phase.

D. Analysis of Plasma FSH Concentrations Throughout the Porcine Estrous Cycle

1. Introduction. Little is known about the temporal fluctuations of plasma FSH during the reproductive cycle of the major domestic species. Preliminary studies of FSH concentrations during the estrous cycle of the ewe (L'Hermite et al., 1972) and sow (Rayford et al., 1973) have recently been reported. In the ewe, FSH concentrations were found to be low prior to estrus with FSH peaks occurring during the preovulatory LH surge (day 0), day 1 and day 2. The data for the sow indicate that FSH concentrations do not rise during the preovulatory LH surge; however, an increase in FSH concentration was observed between days 2 and 3.

This study was initiated to critically analyze FSH concentrations during the porcine estrous cycle and evaluate those fluctuations in relation to LH concentrations obtained during the same period.

2. Materials and Methods. Indwelling cannulae were inserted into the vena cava of 12 nulliparous Yorkshire sows after they had completed at least 1 estrous cycle (18 to 22 days). Six of the 12 sows, hereafter designated as sham treated, were laparotomized in order to inspect the reproductive tract. The animals were housed in special holding stalls in the Large Animal Laboratory at the University of Maryland so that temperature and light could be controlled. Blood samples were collected at 6 hr intervals (beginning at 0600 hrs) over a period of one estrous cycle; however, samples collected during the first 3 days after surgery were not included in the data analysis. Plasma was removed by centrifugation and stored frozen until assayed by radioimmunoassay (Rayford et al., 1973).

The mean and standard deviation of the basal population of hormone concentrations were computed with the truncation procedure (sub-heading B of Section IV., Results). All hormone fluctuations were evaluated in re-

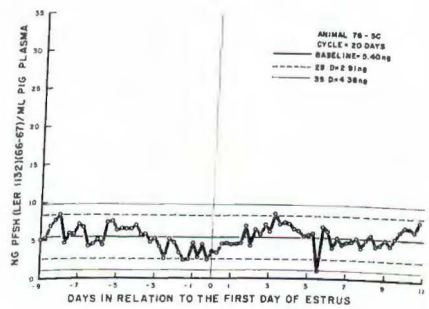
lation to the mean of the basal population.

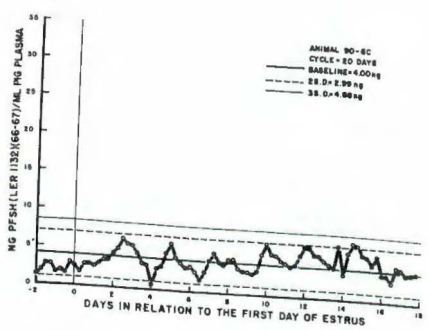
3. Results. The profiles of FSH concentrations in the 5 control swine are shown in figure 8. The largest and most consistent plasma FSH peak occurred between days 2 and 3 reaching an average concentration of 9.82±2.68 ng/ml (table 18). During this time, FSH concentrations remained above baseline for a period of 62.37±7.72 hrs before returning to baseline (5.38±1.55 ng/ml, table 19). The initial rise of the postovulatory FSH (PoOvFSH) peak coincided with the initial rise of the preovulatory LH (PrOvLH) peak, but FSH did not reach maximum concentrations until 59.19±11.86 hrs (2.46±.49 days, table 20) after the preovulatory LH peak.

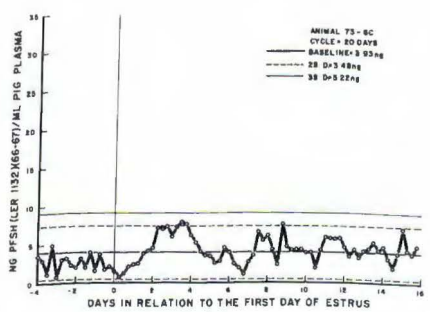
The least squares regression equations of FSH concentrations in the ascending and descending halves of the postovulatory FSH peak fitted a straight line when the FSH concentrations were plotted as a function of time (figure 9). The slopes of the ascending (Y $\log_{10}$=.918±.007X, r=.620**, df=23) and descending (Y $\log_{10}$=.906-.002X, r=.170, df=34) halves of the postovulatory FSH peaks were significantly different (t=3.16**, df=57). The slope of the ascending half of the PoOvFSH peak was significantly different from 0 (ascending t=3.77**, df=23); however, the slope on the descending half of the PoOvFSH peak was not significantly different (P>.05) from 0 (descending t=1.03, df=34). The descending slope of the PoOvFSH peak was not significantly different from zero due to the slow and variable rate at which these FSH concentrations returned to baseline.

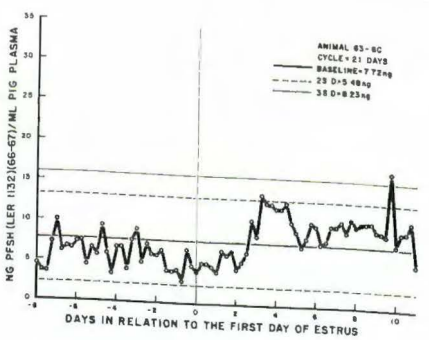
FSH concentrations in the ascending half of the PoOvFSH peak rose from baseline to peak in 25.67 hrs (figure 9). The FSH concentrations on the descending side of the PoOvFSH peak required an estimated 92.69 hrs to decrease from peak to baseline (time estimates computed with least squares regression equation). Graphical estimates of the time required for baseline FSH concentration to reach maximum concentration and return to base-

Figure 8. Temporal pattern of plasma FSH concentrations during the estrous cycle of 5 control swine. All blood collections begin at 0600 hrs on the third day of cannulation.


ANIMAL 76 - 5C
CYCLE = 20 DAYS
BASELINE= 5.40ng
28 D=2.91ng
35 D=4.38ng
NG PFSH (LER 1132)(66-67)/ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 90 - 6C
CYCLE = 20 DAYS
BASELINE=4.00ng
28 D=2.99 ng
35 D=4.86ng
NG PFSH (LER 1132)(66-67)/ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 73 - 6C
CYCLE = 20 DAYS
BASELINE= 3.93ng
28 D=3.48ng
35 D=5.22ng
NG PFSH (LER 1132)(66-67)/ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 63 - 8C
CYCLE = 21 DAYS
BASELINE= 7.72ng
28 D=5.48ng
35 D=8.23ng
NG PFSH (LER 1132)(66-67)/ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS

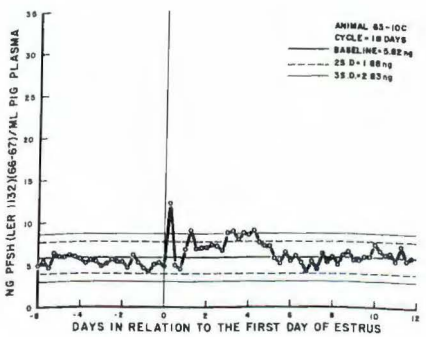
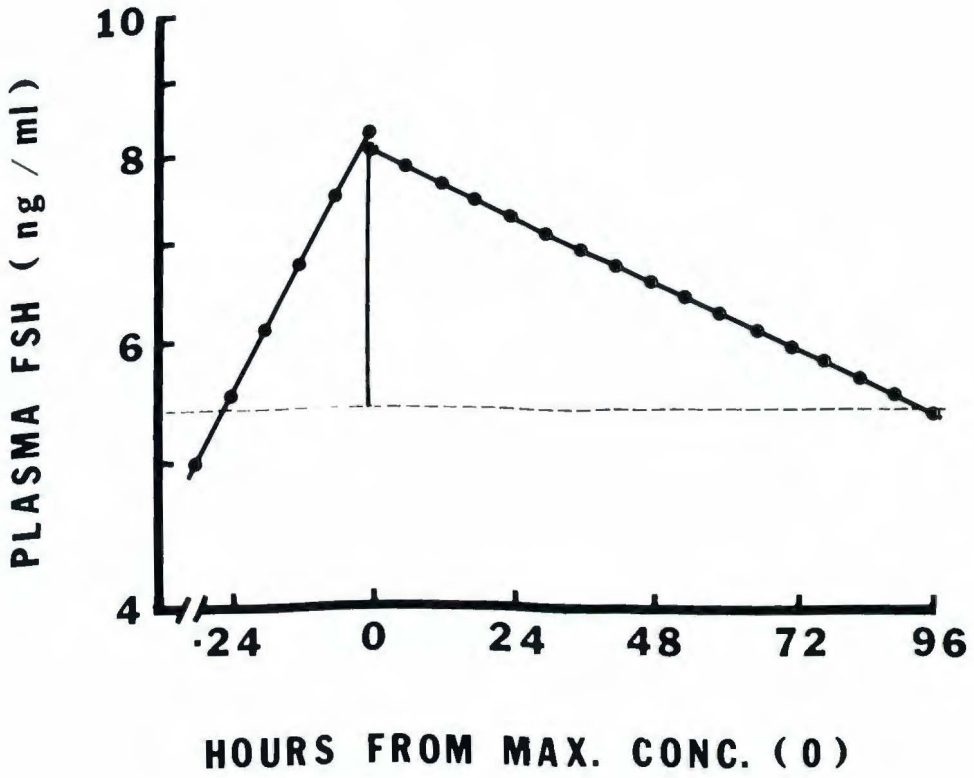

ANIMAL 63 - 10C
CYCLE = 18 DAYS
BASELINE= 5.82 ng
25 D= 1.88 ng
35 D.= 2.83 ng
NG PFSH (LER 1132)(66-67)/ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS

Figure 9. Least squares regression analysis of plasma FSH concentrations in the ascending and descending halves of the postovulatory FSH peak. The slopes of the ascending (Y $\log_{10}$=.918+.008X, r=.620**) and descending (Y $\log_{10}$=.906-.002X, r=.17) halves of the postovulatory peak were significantly different (t=3.16**, df=57). Plasma FSH reached maximum concentration in 25.67 hrs and returned to baseline (----) 92.69 hrs after maximum concentration had been reached.


10
8
6
4
PLASMA FSH (ng / ml)
-24
0
24
48
72
96
HOURS FROM MAX. CONC. (0)

line were 21.92±14.73 and 47.62±20.37 hrs, respectively (time estimates calculated from variate-time graph data, figure 8).

An average of 1.4±.55 mid-cycle FSH peaks occurred between days 8 to 14. These peaks reached a maximum concentration of 10.02±4.23 ng/ml and returned to baseline in 17.01±6.01 hrs (table 18). The average mid-cycle FSH ($\bar{x}$MCFSH) peaks occurred approximately 10.36±2.48 days (table 20) after the preovulatory LH peak; 7.86±2.90 days after the postovulatory FSH peak and approximately 9.52±2.72 days before the next preovulatory LH peak.

A randomized complete block analysis of variance of hormone concentrations during the 4 collection periods indicated a significant effect of block ($P<.01$) and time of day ($P<.05$) (table 21). Mean FSH concentration at the 2400 hr collection interval was significantly greater ($P<.05$) than the mean FSH concentration at the 1800 hr collection interval (figure 10). FSH concentrations which were significantly greater than baseline were distributed uniformly among the 4 collection intervals ($\chi^2=2.0$, df=3, $P>.05$).

The distribution of FSH concentrations which are significantly greater ($P<.05$) than baseline is shown in figure 11 in relation to their occurrence within the estrous cycle. A randomized block analysis of variance of FSH concentrations exceeding 2 standard deviations from the mean of the basal population indicated a nonsignificant effect ($P>.05$) of day of cycle (table 22). The number of concentrations found to be significantly greater than baseline during days 3 to 6 was not significantly greater ($P>.05$) than the number of FSH concentrations excluded during the other 4 collection intervals.

The analysis of sample means indicated no significant differences between control and sham operated animals at the 5% level of signifi-

Figure 10. The mean and standard deviation of plasma FSH concentrations at the 0600, 1200, 1800 and 2400 hr collection periods during the porcine estrous cycle. The mean plasma FSH concentration at the 2400 hr collection period was significantly greater ($P<.05$) than the mean of the 1800 hr collection period.

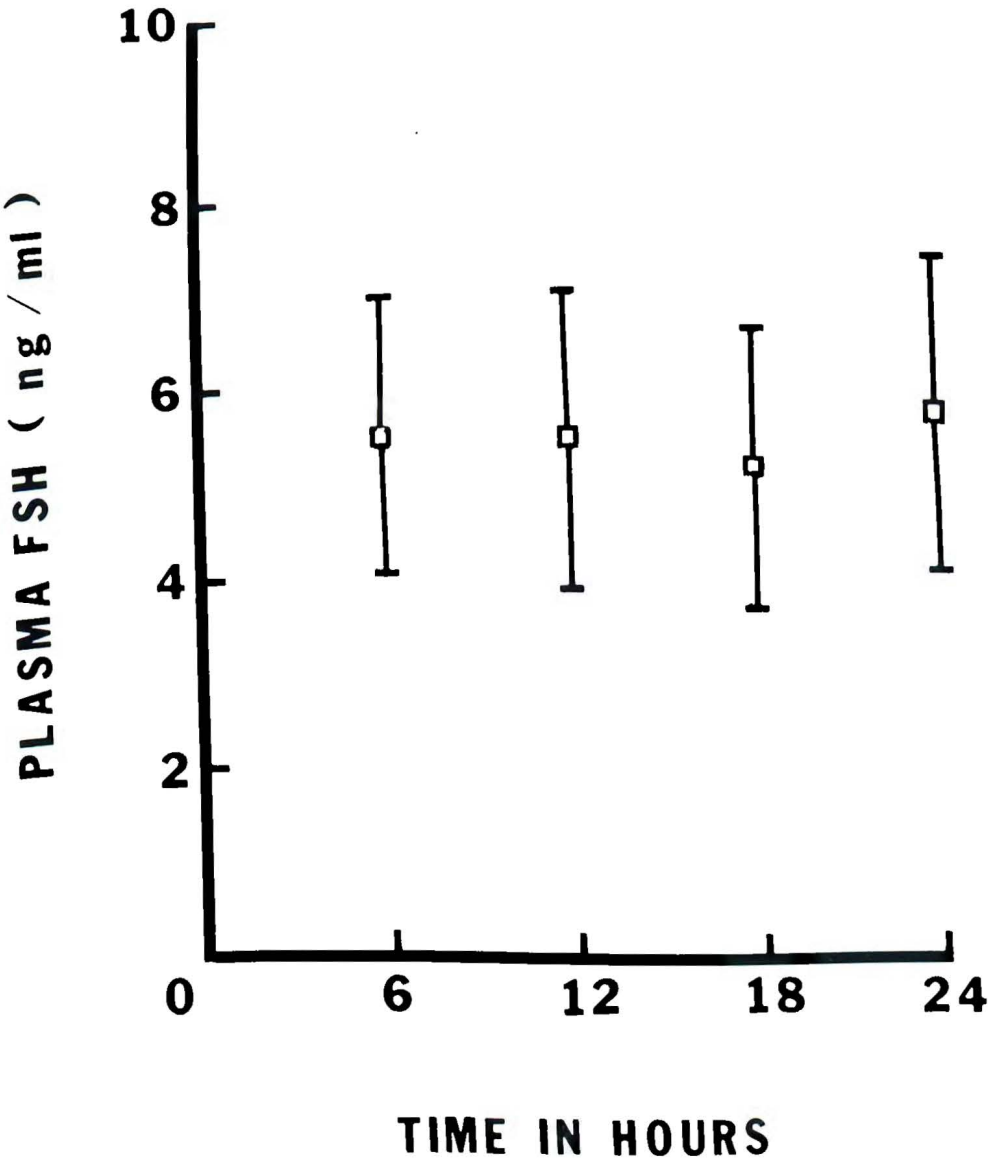
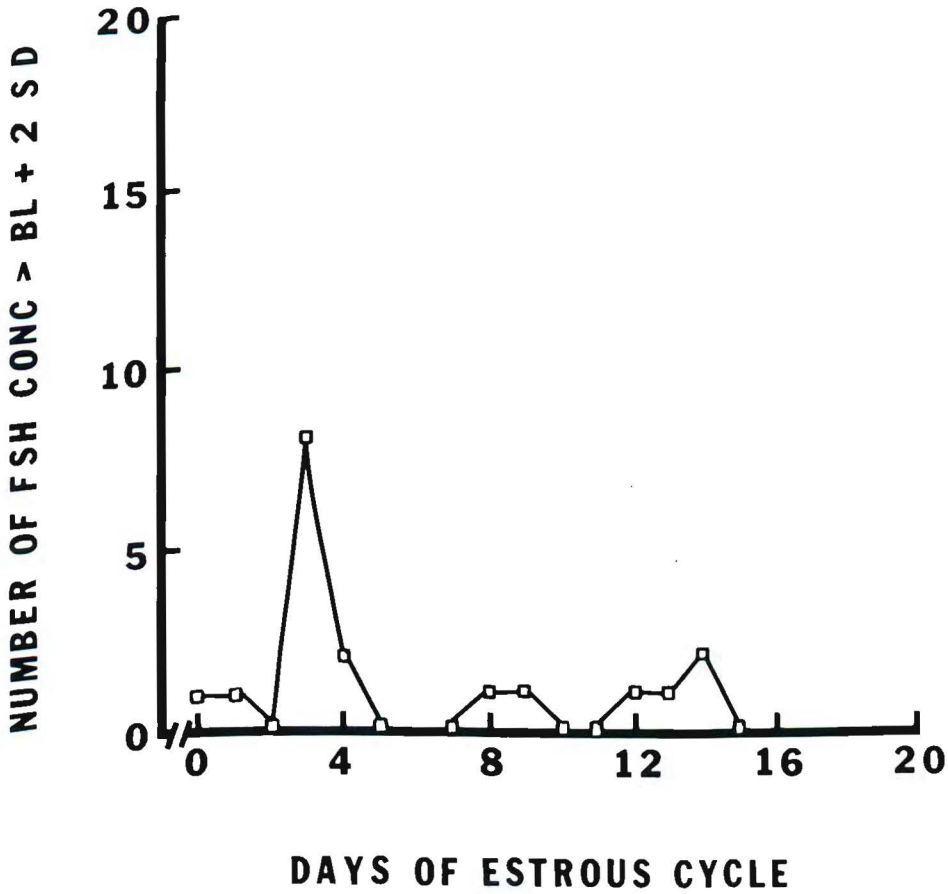

10
8
6
4
2
0
6
12
18
24
PLASMA FSH (ng / ml)
TIME IN HOURS

Figure 11. The distribution of plasma FSH concentrations which are significantly greater than baseline, in relation to the day of the estrous cycle.


NUMBER OF FSH CONC > BL + 2 SD
20
15
10
5
0
0
4
8
12
16
20
DAYS OF ESTROUS CYCLE

cance (tables 18 to 20). Since no significant differences were observed between the means of the control and sham treated animals, future statistical analyses were based on the grand mean of these 2 treatments.

The concentration of FSH in the PoOvFSH peak (pooled data from control and sham operated animals) was not significantly different (P>.05) from the FSH concentrations in the $\bar{x}$MCFSH peaks (PoOvFSH 9.82±2.68 ng/ml *vs* $\bar{x}$MCFSH 10.02±4.23 ng/ml, t=.89, df=14); however, FSH concentrations remained elevated above baseline for a significantly greater (P< .05) period of time during the PoOvFSH peak (PoOvFSH 62.37±7.72 hrs *vs* $\bar{x}$MCFSH 17.01±6.01 hrs, t=6.23**, df=14, table 18).

4. Discussion. An analysis of plasma concentrations obtained at 6 hr intervals during estrus did not indicate the presence of any consistent, significant or concomitant rise in FSH concentration during the preovulatory LH surge (figure 8). A significant rise in plasma FSH during the preovulatory LH surge was observed in only 1 of 10 animals; however, minor nonsignificant fluctuations were observed in 6 other animals. The absence of a significant FSH peak during the preovulatory LH surge in the pig is consistent with the earlier observations of Rayford *et al*. (1973). Although plasma FSH concentrations have not been studied extensively, a peak which coincides with the preovulatory LH surge has been found in sheep (L'Hermite *et al*., 1972), mice (Kovacic and Parlow, 1972), chimpanzee (Howland *et al*., 1971) and humans and rats (Gay *et al*., 1970).

The absence of a significant FSH peak during the preovulatory LH surge was unexpected since a 60% decrease in pituitary FSH content has been observed in the pig during estrus (days 0 to 3, Parlow *et al*., 1964). Low plasma FSH concentrations during estrus may be due to uptake of FSH by the follicles or an increased rate of hormone catabo-

lism by the liver (Song et al., 1969). These conclusions seem justified since plasma FSH reached maximum concentrations during or after ovulation (days 2 to 3) while pituitary content remains low from day 0 to day 3. Since pituitary content decreases from days 0 to 3 and plasma FSH concentrations do not rise significantly until days 2 to 3, the presumably increased FSH secretion must be associated with an increased rate of hormone removal in order to maintain plasma FSH at the low follicular phase concentrations observed. The increase in FSH concentration during and after ovulation may reflect a decrease in FSH utilization by the follicles as a result of their conversion into corpora lutea during ovulation. Additional support for the follicular FSH utilization hypothesis can be found in the fact that the pig ovulates 5 to 15 times more follicles than the other animals in which a preovulatory FSH peak has been observed. The larger number of developing follicles would require more FSH, thus decreasing the probability of finding a significant concomitant preovulatory rise of FSH in the pig.

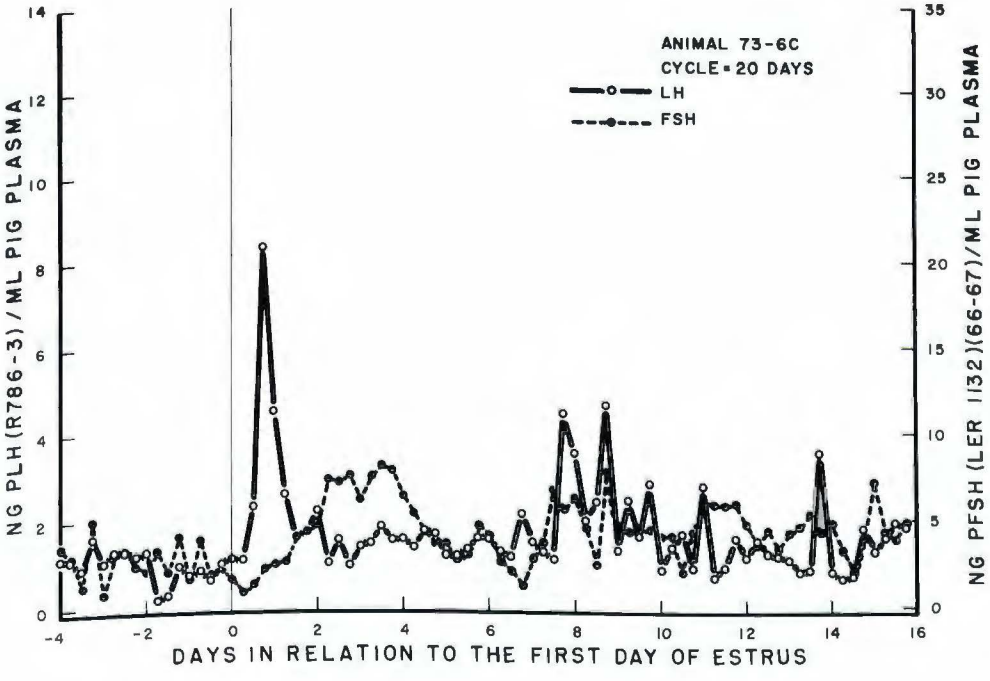
The only major difference between porcine and ovine plasma FSH profiles is the presence of the preovulatory FSH rise in sheep. Plasma FSH concentrations also increase during or after the expected time of ovulation in the sheep (days 1 and 2). FSH concentration remains at intermediate levels for the duration of the luteal phase before declining to their lowest concentrations during the follicular phase of the cycle. The low FSH concentrations observed during the follicular phase of the cycle may be due to an initial inhibition of FSH secretion by rising estrogen concentrations (Chakraborty et al., 1972) as well as increased FSH utilization by the growing follicles.

The increased concentrations of plasma FSH observed during days 2 to 3, may serve as the stimulus for accelerated follicular growth during

days 2 to 10 (Green, 1950; Robinson and Nalbandov, 1951; Parlow et al., 1964). According to the data of Green (1950) and Robinson and Nalbandov (1951) this growth was most pronounced between days 8 to 13. In conjunction with this accelerated follicular growth, minor plasma estrogen peaks were observed between days 4 and 8 (Henricks et al., 1972). These small increases in plasma estrogen may be responsible for the luteal phase LH peaks observed during days 7 to 13 (figure 12).

The analysis of plasma FSH concentrations at the 4 collection periods (0600, 1200, 1800 and 2400 hr) indicated that FSH concentrations were higher at the 2400 hr collection period than at the 1800 hr collection period. The daily rhythmicity of plasma FSH was not very dramatic due to the low, broad FSH fluctuations. These observations were similar to those which have been reported in other species (Rubin et al., 1972; Kapen et al., 1973).

Figure 12. The temporal pattern of plasma LH and FSH concentrations in control animal 73-6C, during the porcine estrous cycle. All data collections begin at 0600 hrs on the third day of cannulation.


ANIMAL 73-6C
CYCLE = 20 DAYS
LH
FSH
NG PLH (R786-3) / ML PIG PLASMA
NG PFSH (LER 1132)(66-67)/ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS
0
2
4
6
8
10
12
14
-4
-2
0
2
4
6
8
10
12
14
16
0
5
10
15
20
25
30
35

E. Analysis of Plasma PRL Concentrations Throughout the Porcine Estrous Cycle

1. Introduction. The physiological importance of prolactin in the control of the bovine, ovine and porcine estrous cycle appears to be negligible; however, minor plasma hormone fluctuations do occur in these species (Hansel and Echternkamp, 1972). Plasma prolactin concentrations are elevated during estrus in the cow (Swanson and Hafs, 1971) and sheep (Davis et al., 1971). In addition to the preovulatory increase in ovine plasma prolactin, Davis et al. (1971) also found prolactin concentrations to rise at 3 to 4 day intervals during the cycle. Porcine plasma prolactin concentrations have not been reported, but Threlfall et al. (1972) have observed a decrease in prolactin content in the porcine pituitary during estrus.

This study was initiated to determine the temporal pattern of plasma prolactin concentrations during the porcine estrous cycle and evaluate those fluctuations in relation to LH and FSH concentrations obtained during the same period.

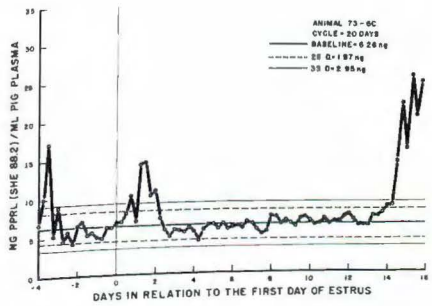
2. Materials and Methods. Indwelling cannulae were inserted into the vena cava of 12 Yorkshire sows after they had completed at least 1 estrous cycle (18 to 22 days). Six of the 12 sows, hereafter designated as sham treated, were laparotomized in order to inspect the reproductive tract. The animals were housed in special holding stalls in the Large Animal Laboratory at the University of Maryland so that temperature and light could be controlled. Blood samples were collected at 6 hr intervals (beginning at 0600 hrs) over a period of 1 estrous cycle; however, blood samples collected during the first 3 days after surgery were not included in the data analysis. Plasma was removed by centrifugation and stored frozen until assayed by radioimmunoassay (Brinkley et al., 1972).

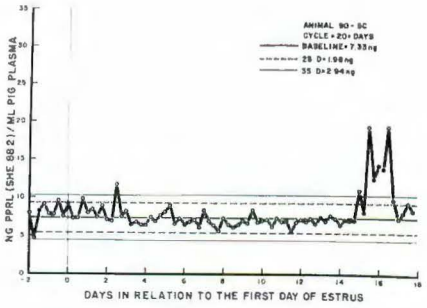
The mean and standard deviation of the basal population of hormone concentrations were computed with the truncation procedure (sub-heading B of Section IV., Results). All hormone fluctuations were evaluated in relation to baseline.

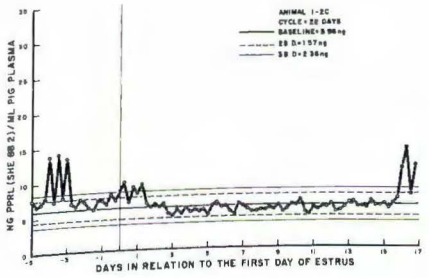
3. Results. The greatest increase in plasma prolactin concentrations occurred during the beginning of the follicular phase of the porcine estrous cycle (figure 13). An average of 3.17±1.60 follicular phase prolactin (FoPRL) peaks occurred between days 14 to 19. These peaks reached a maximum concentration of 13.70±4.02 ng/ml and returned to baseline (6.16±.94 ng/ml, table 23) in 19.49±2.92 hrs (table 24). The follicular phase prolactin peaks reached maximum concentrations 15.59 ±1.01 days (table 25) after the preovulatory LH (PrOvLH) peak and 13.23± 1.47 days after the postovulatory FSH (PoOvFSH) peak. They appeared approximately 4.05±1.10 days before the preovulatory LH peak and 6.84±.45 days before the postovulatory FSH peak.

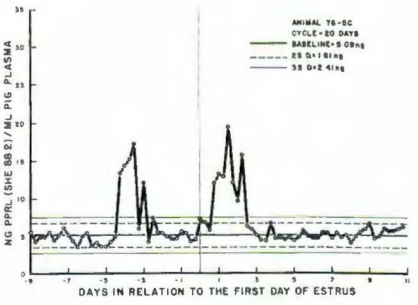
The equations representing prolactin concentrations in the ascending ($Y \log_{10}=1.144+.015X$, $r=.670^{**}$, $df=47$) and descending ($Y \log_{10}=1.144-.025X$, $r=-.560^{**}$, $df=28$) halves of the follicular phase prolactin peaks were estimated by least squares regression analysis (figure 14). The rate of change in prolactin concentration (slope) was significantly greater on the descending side of the FoPRL peak than on the ascending half of the peak ($t=5.99^{**}$, $df=75$). The geometry of the FoPRL peak was notably different from that seen in the major LH and FSH peaks since the greatest rate of change in LH and FSH hormone concentrations occurred on the ascending half of the peak. An estimated 23.86 hrs were required for plasma prolactin to rise from baseline to maximum concentration, but PRL concentrations declined from maximum concentration to baseline in only 14.08 hrs. Least squares regression analysis of prolactin concentrations in the

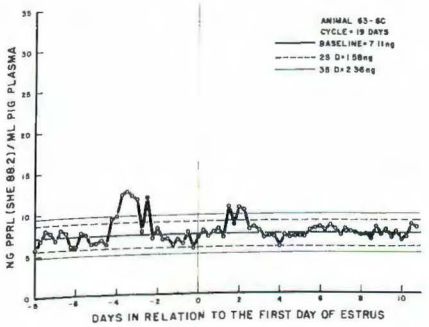
Figure 13. Temporal pattern of plasma prolactin concentrations during the estrous cycle of 6 control swine. All blood collections begin at 0600 hrs on the third day of cannulation.


ANIMAL 73 - 6C
CYCLE = 20 DAYS
BASELINE = 6.26 ng
25 D = 1.87 ng
35 D = 2.95 ng
NG PPRL (SHE 88.2) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 90 - 8C
CYCLE = 20+ DAYS
BASELINE = 7.33 ng
25 D = 1.98 ng
35 D = 2.94 ng
NG PPRL (SHE 88.2) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 1-2C
CYCLE = 22 DAYS
BASELINE = 5.98 ng
25 D = 1.57 ng
35 D = 2.36 ng
NG PPRL (SHE 88.2) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 76-5C
CYCLE = 20 DAYS
BASELINE = 5.09 ng
25 D = 1.61 ng
35 D = 2.41 ng
NG PPRL (SHE 88.2) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS


ANIMAL 63-8C
CYCLE = 19 DAYS
BASELINE = 7.11 ng
25 D = 1.58 ng
35 D = 2.36 ng
NG PPRL (SHE 88.2) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS

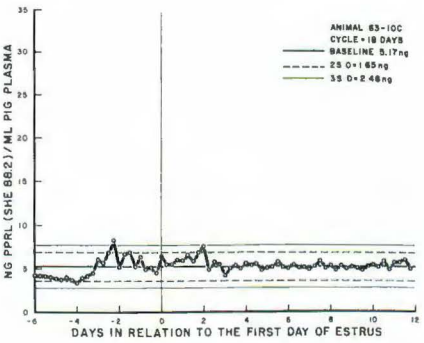
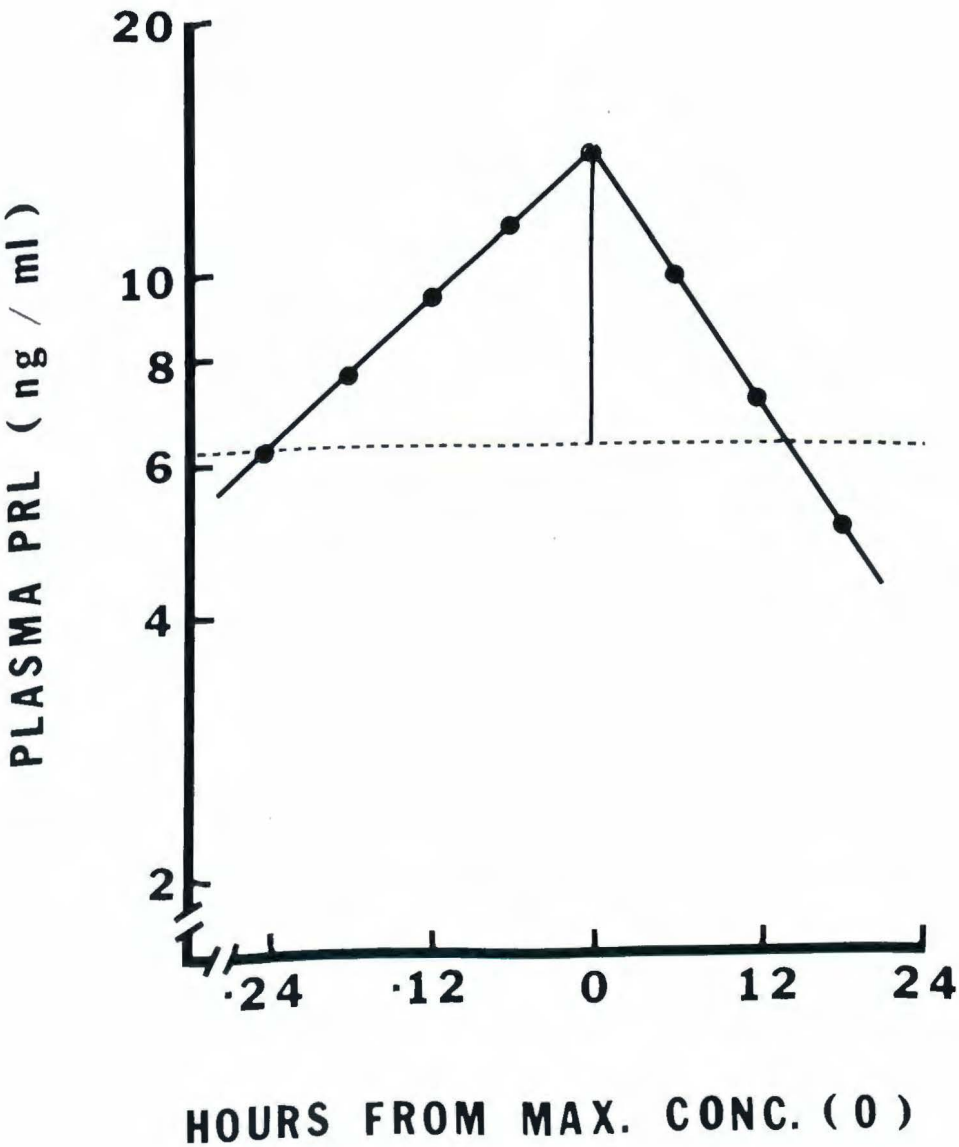

ANIMAL 63-10C
CYCLE = 18 DAYS
BASELINE 5.17 ng
25 D = 1.65 ng
35 D = 2.48 ng
NG PPRL (SHE 88.2) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS

Figure 14. Least squares regression analysis of plasma prolactin concentrations in the ascending and descending halves of the follicular phase peaks. The slope of the ascending (Y $\log_{10}$=1.144+.015X, r=.670**) and descending (Y $\log_{10}$=1.144-.025X, r=.560**) halves of the follicular phase peak were significantly different (t=5.99**, df=75). Plasma concentrations rose from baseline to maximum concentration in 28.36 hrs and then returned to baseline (----) 14.08 hrs after maximum plasma concentration had been reached.


20
10
8
6
4
2
PLASMA PRL (ng / ml)
·24
·12
0
12
24
HOURS FROM MAX. CONC. (0)

preovulatory prolactin (PrOvPRL) peak indicated that the slopes of the ascending and descending halves of this peak were not significantly different from 0.

In addition to the FoPRL peaks, an average 2.50±1.05 preovulatory prolactin peaks occurred either before or after the preovulatory LH surge. The preovulatory prolactin peaks rose from baseline to a maximum concentration of 10.30±1.88 ng/ml and declined to baseline over a period of 20.21±4.18 hrs (table 24). The PrOvPRL peaks reached a maximum concentration 9.95±9.54 hrs after the PrOvLH peak had reached maximum concentration and 52.49±11.50 hrs before PoOvFSH peak had reached maximum concentration. The preovulatory prolactin peaks occurred approximately 4.91±.75 days after the FoPRL peaks (table 25). The average interval between adjacent prolactin peaks was 20.72±6.80 hrs.

Time of day did not have a significant effect ($P>.05$) on prolactin concentration (figure 15, table 26). Plasma prolactin concentrations which were significantly greater than baseline were not abnormally distributed ($P>.05$) within the 4 collection intervals ($\chi^2=1.55$, df=3).

Plasma prolactin concentrations which were significantly greater than baseline are shown in figure 16 in relation to their occurrence within the porcine estrous cycle. A randomized block analysis of variance of prolactin concentrations exceeding 2 standard deviations from the mean of the baseline population indicated a significant effect of day of cycle ($P<.01$). The number of concentrations excluded during days -1 to 2 and 15 to -2 was significantly greater ($P<.01$) than the number of concentrations excluded during the remainder of the estrous cycle (table 27).

The mean plasma prolactin concentration in the preovulatory prolactin peaks (control + sham treated animals) was not significantly

Figure 15. The mean and standard deviation of plasma PRL concentrations at the 0600, 1200, 1800 and 2400 hr collection periods during the porcine estrous cycle. The mean plasma PRL concentrations at the 4 collection periods were not significantly different ($P>.05$).

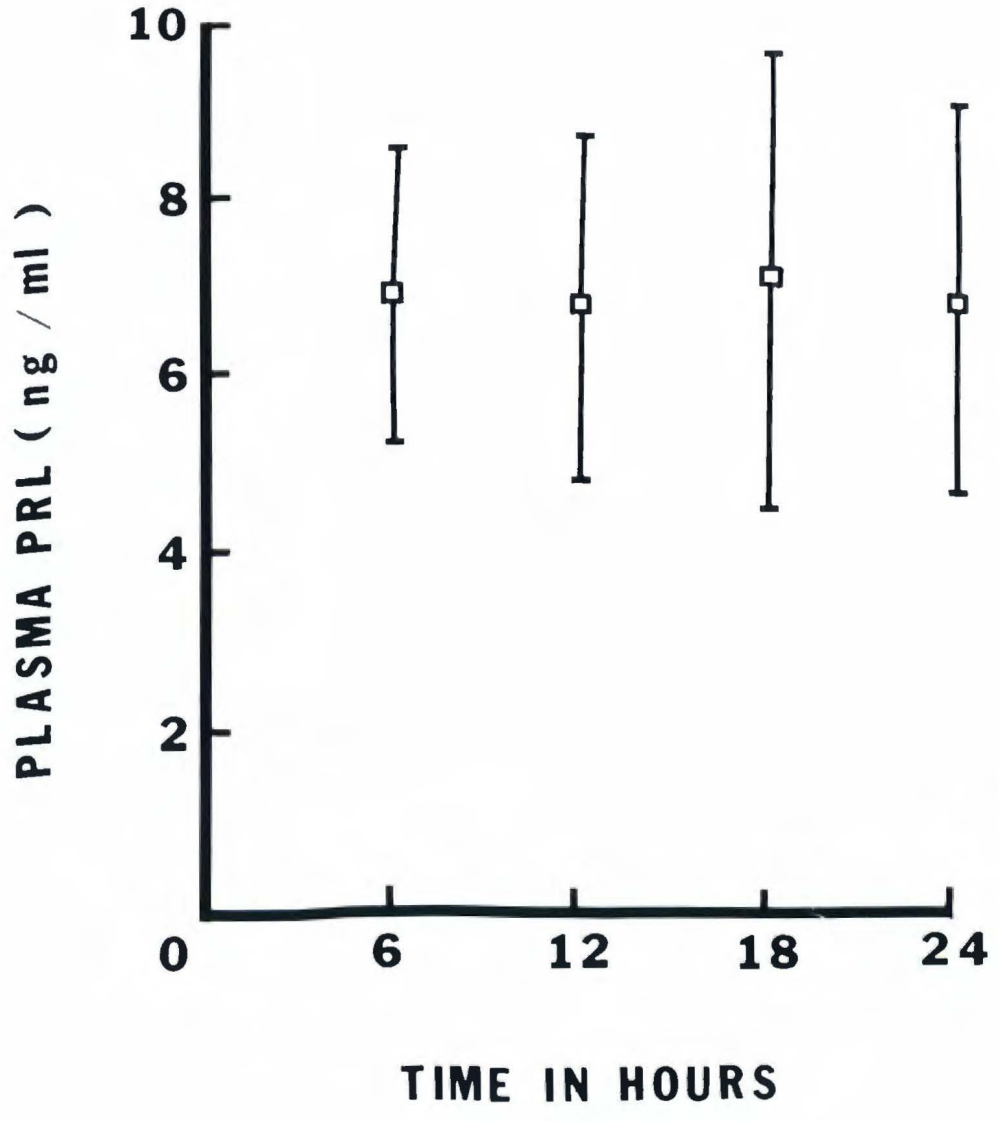
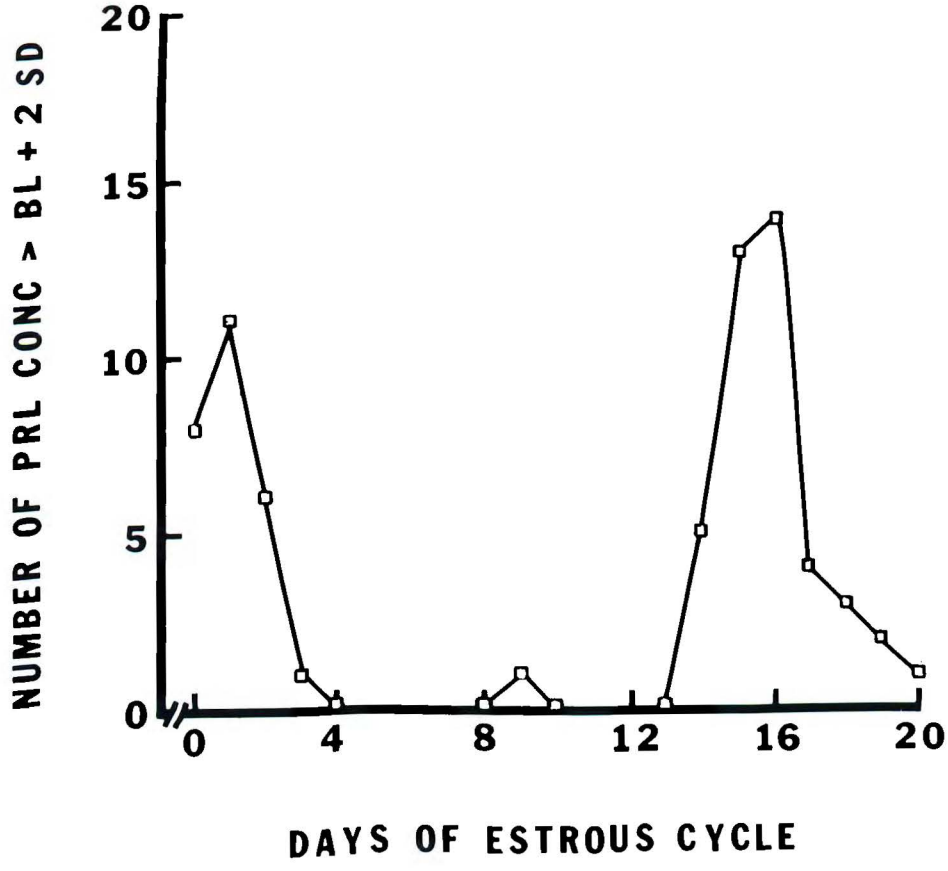

10
8
6
4
2
0
6
12
18
24
PLASMA PRL (ng / ml)
TIME IN HOURS

Figure 16. The distribution of plasma PRL concentrations which were significantly greater than baseline, in relation to the day of the estrous cycle.


NUMBER OF PRL CONC > BL + 2 SD
20
15
10
5
0
0
4
8
12
16
20
DAYS OF ESTROUS CYCLE

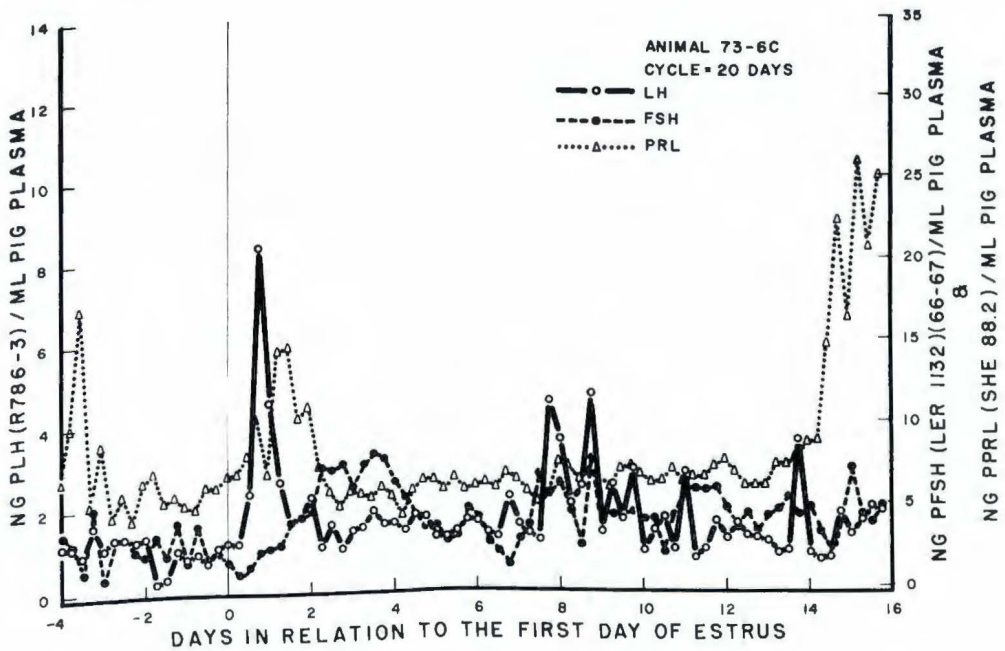
different (P>.05) from the mean prolactin concentration in the follicular phase prolactin peaks (PrOvPRL 11.49±2.97 ng/ml vs FoPRL 14.28±4.27 ng/ml, t=1.85, df=22). The length of time that prolactin concentrations in the PrOvPRL and FoPRL peaks remained elevated above baseline was not significantly different (PrOvPRL 20.54±4.02 hrs vs FoPRL 18.98±3.30 hrs, t=1.04, df=22, table 24).

4. Discussion. An analysis of plasma prolactin concentrations during estrus (figure 13) indicated that small elevations in prolactin concentrations occur before, during and after the preovulatory LH surge in the pig. These increases in plasma prolactin occur at a time when pituitary prolactin content is decreasing (Threlfall et al., 1972). Elevated plasma prolactin concentrations during estrus were presumably due to the elevated estrogen concentrations which preceded the preovulatory LH surge since estrogen has been shown to stimulate the synthesis and release of prolactin in many species (Meites et al., 1963). An increase in plasma prolactin concentration during estrus has also been observed in the cow (Swanson and Hafs, 1971), sheep (Davis et al., 1971) and rat (Gay et al., 1970).

The temporal fluctuations of LH and FSH during the luteal phase were markedly different from plasma prolactin concentrations which were maintained at basal levels throughout this period (figure 17). Low plasma estrogen concentrations superimposed upon elevated plasma progestogen concentrations do not appear to be conducive to prolactin secretion.

The largest and most consistent plasma prolactin peaks occurred during the end of the luteal phase and the beginning of the follicular phase of the porcine estrous cycle (figure 13). Rising plasma estrogen concentrations may facilitate the increase in plasma prolactin concentrations in a manner similar to that observed during parturition (Fell et al.,

Figure 17. The temporal patterns of plasma LH, FSH and PRL concentrations in control animal 73-6C, during the porcine estrous cycle.


ANIMAL 73-6C
CYCLE = 20 DAYS
LH
FSH
PRL
NG PLH(R786-3) / ML PIG PLASMA
NG PFSH (LER 1132)(66-67)/ML PIG PLASMA
&
NG PPRL (SHE 88.2) / ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS

1972). A least squares regression analysis of prolactin concentrations in the ascending half of the follicular prolactin peak indicated that more time was required for prolactin concentrations to rise from baseline to maximum concentration than to decline from maximum concentration to baseline (figure 14). The profile of prolactin concentrations during the follicular phase peak was distinctly different from those observed for LH (figure 5) and FSH (figure 9). The peculiar appearance of plasma prolactin concentrations has not drawn the attention of other investigators; however, plasma prolactin peaks in the rat (Gay et al., 1970) and sheep (Fell et al., 1972) appear to follow a similar pattern.

The physiological importance of elevated prolactin concentrations during the estrous cycle has not been resolved. Astwood (1941) discovered that prolactin was capable of maintaining the corpora lutea in rats; however, it has not been established whether prolactin exerts a luteotropic effect during the normal estrous cycle. Prolactin has been tested for its luteotropic action in other species and was found to be ineffective in maintaining corpora lutea in guinea pigs, rabbits, cats, dogs, sheep, pigs, cows and women (Nalbandov, 1970). Recently, Wuttke and Meites (1971) have suggested that the normal rise of rat prolactin on the day of proestrus and continuing into estrus serves to induce luteolysis of the previous crop of corpora lutea formed during each cycle.

As in the rat, insufficient data exists to determine the importance of prolactin during the porcine estrous cycle. Low prolactin concentrations observed during the luteal phase (figures 13 and 16) suggest that prolactin is not important in stimulating progesterone secretion. However, it is possible that prolactin may be stored in the ovulatory follicle, as previously demonstrated for LH in the sheep, (Bjersing et al.,

1972) and thus play a part in the subsequent development and function of the corpus luteum. The rise in follicular phase plasma prolactin concentration occurs at a time when the corpora lutea are known to be regressing. This suggests that the rise in follicular phase plasma prolactin may function as a luteolytic agent in a manner similar to that previously described for the rat (Wuttke and Meites, 1971). This hypothesis may warrent further consideration since a significant, positive correlation exists between estrous cycle length and the time interval between the preovulatory LH peak and the average follicular phase prolactin peak ($r=.729^{**}$, $df=10$).

The analysis of plasma prolactin concentrations at 6 hr intervals during the estrous cycle failed to indicate any circadian rhythmicity in plasma prolactin concentrations. Biological fluctuations in plasma prolactin concentration have been reported previously in man (Sassin et al., 1973; Parker, et al., 1973) and the rat (Gay et al., 1970).

The extremely stable prolactin baseline observed during the estrous cycle supports the hypothesis that the animals were not stressed during blood removal since stressful stimuli have been shown to elevate serum prolactin concentrations in many species (Butler et al., 1971; Raud et al., 1971; Johke et al., 1971).

F. Analysis of Plasma LH, FSH and PRL Concentrations in Ovariectomized Swine

1. Introduction. Many investigators have reported increases in pituitary and serum gonadotropin concentrations as a result of ovariectomy. According to classic reproductive endocrinology, the ovary produces steroids which inhibit the secretion of pituitary gonadotropins. Removal of this inhibitory feedback by ovariectomy allows LH and FSH concentrations to rise while prolactin concentrations remain unchanged. Recent evidence indicates that estradiol may be the principal steroid responsible for the control of gonadotropin secretion in the rat (Kobayashi et al., 1969), sheep (Goding et al., 1969), cow (Hobson and Hansel, 1972) and human (Swerdloff and Odell, 1969).

The effect of ovariectomy on pituitary and serum concentrations of LH and FSH in the pig has not been studied extensively. Twenty-five days after ovariectomy, Parlow et al. (1964) found mean LH and FSH concentrations to be approximately 2 times greater than the mean values observed on day 10 of the estrous cycle. Serum LH and FSH concentrations at days 24 and 52 were also found to be significantly greater than the mean serum concentrations obtained from gilts during the estrous cycle (Rayford et al., 1971; Rayford et al., 1973). The present study was initiated to observe the effects of bilateral ovariectomy on sequential plasma LH, FSH and PRL concentrations during the first 24 days of ovariectomy.

2. Materials and Methods. Six nulliparous Yorkshire sows were bilaterally ovariectomized between days 3 and 10 of the estrous cycle after they had completed at least 1 estrous cycle (18 to 22 days). Indwelling cannulae were inserted into the vena cava and blood samples were collected at 6 hr intervals beginning at 0600 hrs and continuing over a period of 23 days. Plasma was removed by centrifugation and stored frozen until

assayed by radioimmunoassay. However, blood samples collected during the first 3 days of cannulation were not included in the data analysis. The 6 unoperated and 6 laparotomized swine described under sub-heading C, D and E of section IV., Results were used as controls. The animals were housed in special holding stalls in the Large Animal Laboratory at the University of Maryland so that temperature and light could be controlled.

The mean and standard deviation of the baseline hormone concentrations were computed with the truncation procedure (sub-heading B of section IV., Results). All hormone fluctuations were evaluated in relation to baseline. Standard factorial analysis of variance was used to evaluate the effect of treatment on LH, FSH and PRL concentrations.

3. Results. Sequential changes in the concentration and pattern of plasma LH, FSH and PRL in representative control, sham and ovariectomized swine are shown in figures 17 to 19. Baseline LH and FSH concentrations in the ovariectomized animals were significantly greater ($P<.05$) than the baseline concentrations in the control or sham treated animals; however, PRL baseline concentration was not affected by treatment (tables 28 to 30). The variance (standard deviation) associated with the basal LH and FSH hormone concentrations also increased in response to ovariectomy (table 31). Student-Newman-Keuls' test of treatment means indicated that only the variance associated with LH concentrations in the ovariectomized animals was significantly different ($P<.05$) from the variance observed in the other treatments (table 32). Due to the elevated baseline and larger variance in the ovariectomized animals, significantly fewer ($P<.05$) LH and FSH concentrations were found to be significantly different from baseline (tables 33 and 34).

Those LH concentrations which were found to be significantly different

Figure 18. The temporal pattern of plasma LH, FSH and PRL concentrations in the sham operated animal, 48-5S, during the porcine estrous cycle. All data collections begin at 0600 hrs on the third day of cannulation.

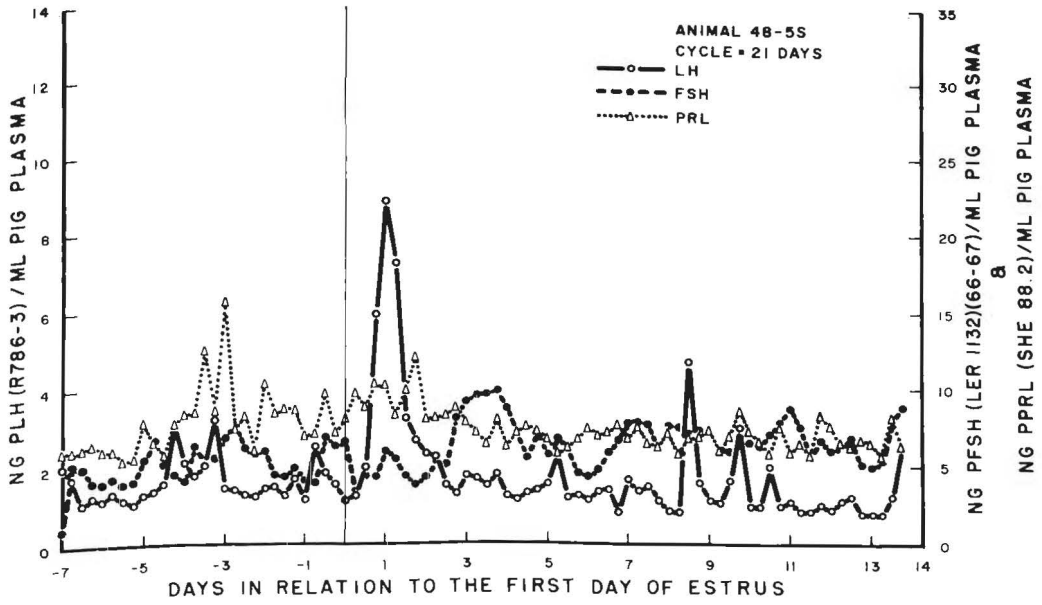
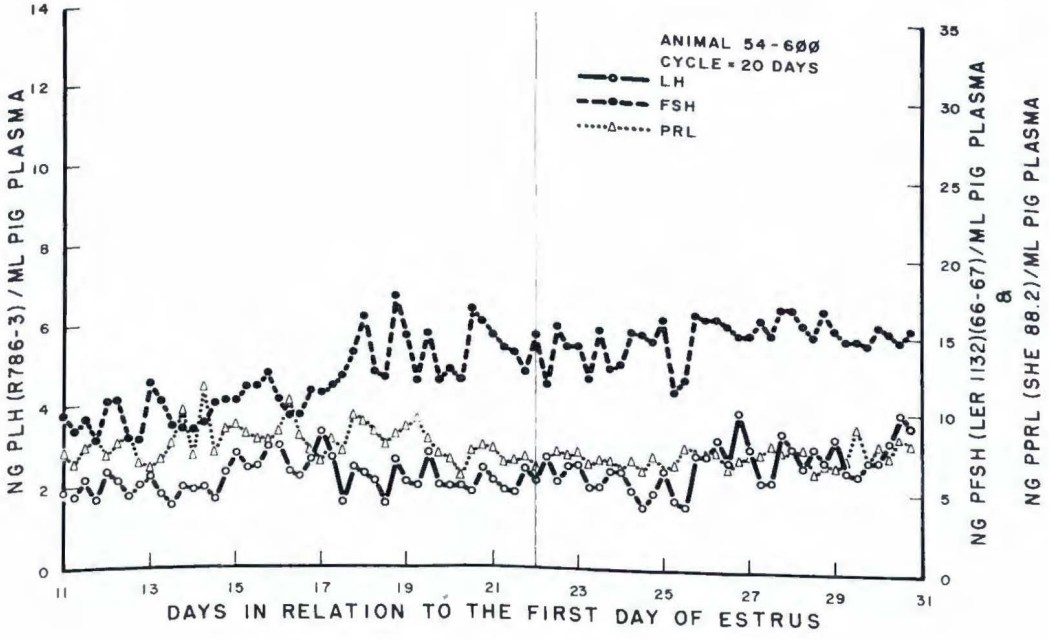

ANIMAL 48-5S
CYCLE = 21 DAYS
LH
FSH
PRL
NG PLH (R786-3) / ML PIG PLASMA
NG PFSH (LER 1132)(66-67)/ML PIG PLASMA
&
NG PPRL (SHE 88.2)/ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS

Figure 19. The temporal pattern of plasma LH, FSH and PRL concentrations between days 4 to 24 after ovariectomy in animal 54-6ØØ. All data collections begin at 0600 hrs on the third day of cannulation.


ANIMAL 54-600
CYCLE = 20 DAYS
LH
FSH
PRL
NG PLH (R786-3) / ML PIG PLASMA
NG PFSH (LER 1132)(66-67)/ML PIG PLASMA
&
NG PPRL (SHE 88.2)/ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS
0
2
4
6
8
10
12
14
0
5
10
15
20
25
30
35
11
13
15
17
19
21
23
25
27
29
31

from baseline, in the ovariectomized animals, were evenly distributed throughout the 20-day collection period. An average of 4.0±2.10 LH peaks rose to a maximum concentration of 3.47±.21 ng/ml plasma and returned to baseline in 14.20±2.46 hrs (table 35). These LH peaks occurred approximately 14.01±2.76 days after ovariectomy. The maximum concentration of LH attained in peaks in ovariectomized pigs (Ovar LH peaks) was significantly less than the maximum LH concentration in the preovulatory LH peak (PrOvLH 7.06±2.40 ng/ml vs Ovar LH 3.47±.21 ng/ml, t=3.59**, df=16). However, the magnitude of the Ovar LH peaks was significantly greater than the concentrations in the class 1 luteal phase peaks (Ovar LH 3.47±.21 ng/ml vs class 1 2.29±.55 ng/ml, t=4.99**, df=10), but not significantly different (P>.05) from the LH concentration of the class 2 luteal phase peaks (Ovar LH 3.47±.21 ng/ml vs class 2 2.86±1.04 ng/ml, t=1.39, df=14, tables 14 and 35). The duration of the Ovar LH peak was significantly less than the duration of the preovulatory LH peak (Ovar LH 14.20±2.46 hrs vs PrOvLH 28.75±4.76 hrs, t=6.96**, df=16) and the class 2 luteal phase peaks (Ovar LH 14.20±2.46 hrs vs class 2 18.61±3.49 hrs, t=2.70*, df=14) but not significantly different from the duration of the class 1 luteal phase peaks (Ovar LH 14.20±2.46 hrs vs class 1 12.65± 1.79 hrs, t=1.54, df=16, tables 14 and 35).

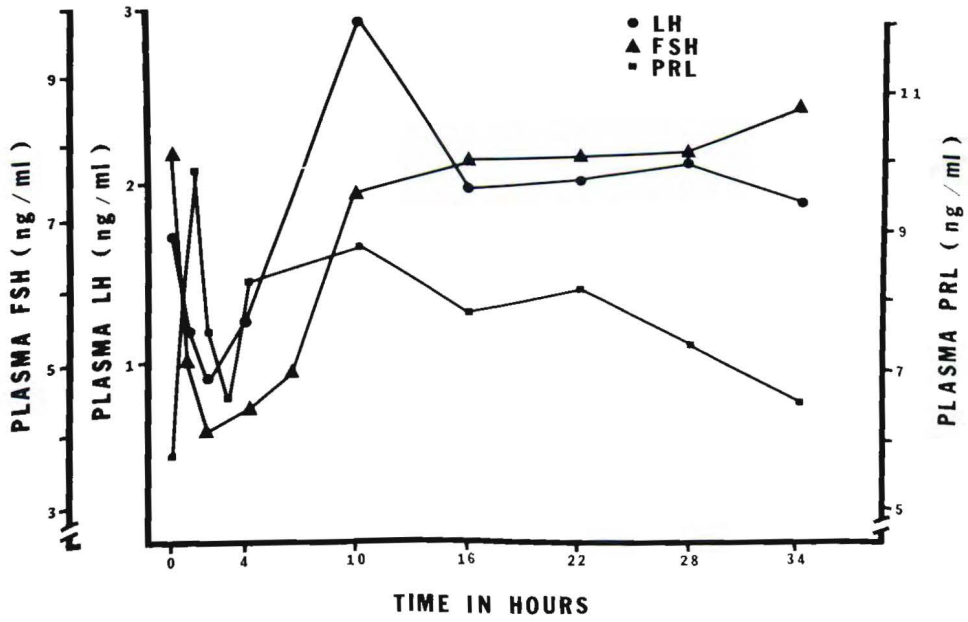
The mean LH concentration on the first day of blood collection (fourth day after ovariectomy) in the ovariectomized animals (1.89±.22 ng/ml) was significantly greater than the mean baseline LH concentration in the 12 control animals (1.19±.25 ng/ml, t=5.80**, df=16). LH concentrations increased slowly from the fourth to the twenty-fourth day after ovariectomy, but the LH concentrations on the last day of sample collection (2.34±.57 ng/ml) were not significantly different (P>.05) from those collected on the fourth day after ovariectomy (1.89±.22 ng/ml, t=1.88,

df=10). Analysis of the LH concentration of samples collected during, and after surgery indicated that LH concentration (3.05±1.59 ng/ml) at 10 hrs after initiation of anesthesia (6 to 8 hrs after removal of ovaries) was significantly greater than the baseline of the control and sham operated animals (1.19±.25 ng/ml, t=4.06**, df=16, figure 20). The mean LH concentration of samples obtained prior to surgery (1.64±.64 ng/ml) was not significantly different from the mean baseline concentration of LH in the control and sham treated animals (1.19±.25 ng/ml, t= 2.15, df=15).

The analysis of blood samples from 5 animals obtained between the fourth and twenty-fourth day after ovariectomy indicated that only 2 FSH concentrations were significantly different from baseline (table 35). Since only 2 significant concentrations were observed, comparisons dealing with the magnitude and duration of FSH concentration in the control and ovariectomized animals were not made.

FSH concentrations were found to increase steadily from the fourth to the twenty-fourth day after ovariectomy. FSH concentrations, on the first day of sample collection (8.56±1.91 ng/ml, fourth day post-ovariectomy), were significantly different from the baseline in the 10 control and sham operated animals (4.76±1.56 ng/ml, t=4.13**, df=13). After 20 days of blood collection, the mean FSH concentrations had nearly doubled (13.08±4.49 ng/ml) and were significantly different from the mean plasma FSH concentration observed on the first day of sample collection (8.56± 1.91 ng/ml, t=2.51*, df=8). FSH concentrations increased in response to ovariectomy in a manner similar to that observed with LH since FSH baseline concentrations in the control and sham treated animals (4.76±1.56 ng/ml) were also found to be significantly different from the mean FSH concentration at 10 hrs after the initiation of surgery (7.30±1.95 ng/ml,

Figure 20. The immediate effects of surgery and ovariectomy on plasma LH, FSH and PRL concentrations in 6 ovariectomized swine. The concentrations of plasma LH and FSH at 10 hrs after initiation of anesthesia were significantly different ($P<.05$) from the baseline hormone concentrations in the 12 control and sham treated swine.


LH
FSH
PRL
PLASMA FSH (ng/ml)
PLASMA LH (ng/ml)
PLASMA PRL (ng/ml)
TIME IN HOURS
0
4
10
16
22
28
34

t=2.74, df=13, figure 20). It should be noted that neither LH nor FSH concentrations in the control animals (control + sham) were significantly different (P>.05) from their respective baseline concentrations at 10 hrs after the initiation of surgery. The initial increase in plasma LH concentration in response to ovariectomy was very precipitous with only a minor increase in hormone concentration being observed during the remainder of the 20-day collection period. In contrast, plasma FSH concentration increased slowly throughout the 20-day collection interval.

An average of 3.17±2.40 PRL concentrations was found to be significantly different from the baseline in the 6 ovariectomized animals. The 2.67±1.97 PRL peaks observed during the 20-day collection interval rose to a maximum concentration of 9.32±2.0 ng/ml and returned to baseline in 16.93 ±5.86 hrs (table 35).

The magnitude of the PRL concentrations which was found to be significantly different from the baseline of the ovariectomized animals (Ovar PRL peaks) was not significantly different from the plasma PRL concentrations associated with the preovulatory prolactin peaks (Ovar PRL 9.32±2.00 ng/ml vs PrOvPRL 11.49±2.97 ng/ml, t=1.61, df=16, tables 24 and 35). However, the magnitude of plasma PRL concentration in the ovariectomized PRL peaks was significantly less than the follicular phase plasma PRL concentrations observed in the control and sham treated swine (Ovar PRL 9.32±2.00 ng/ml vs FoPRL 14.28±4.27 ng/ml, t=2.67*, df=16). The duration of the ovariectomized plasma PRL peaks was not significantly different from the duration of the preovulatory (Ovar PRL 16.93±5.86 hrs vs PrOvPRL 20.54±4.02 hrs, t=1.54, df=16) or the follicular phase prolactin peaks (Ovar PRL 16.93±5.86 hrs vs FoPRL 18.98±3.30 hrs, t=.96, df=16, tables 24 and 35).

Unlike plasma LH and FSH, plasma PRL concentration was not influenced

by ovariectomy. The mean baseline PRL concentration of the control and sham treated animals (6.54±1.29 ng/ml) was not significantly different from the mean baseline concentration in the ovariectomized animals (6.70± 2.10 ng/ml, t=.19, df=16, tables 28 and 29). Mean plasma prolactin concentrations at 10 hrs (8.49±2.28 ng/ml), 4 days (6.64±1.53 ng/ml) and 24 days (7.46±2.17 ng/ml) after ovariectomy were not significantly different (P>.05) from the mean baseline PRL concentration in the control animals (6.16±.94 ng/ml). A sharp increase in plasma prolactin concentration was observed to occur in all animals during the first hr after initiation of anesthesia. This precipitous increase in prolactin presumably was due to surgical stress since it was observed to a varying degree in all animals irrespective of surgical treatment.

The removal of the feedback inhibition of gonadal steroids as a result of ovariectomy did not alter the daily temporal fluctuations of plasma LH concentrations. Mean plasma LH concentration at 2400 hrs was significantly different from mean LH concentrations at 1200 and 1800 hrs (P<.05, tables 36 and 37). The daily pattern of plasma LH concentration in the ovariectomized animals was identical to that observed in the control and sham treated animals. No significant differences (P>.05) were detected in the daily plasma concentrations of FSH and PRL in the ovariectomized swine (tables 36 and 37).

Plasma LH concentrations were found to be distributed uniformly throughout the 5 four-day collection intervals (tables 38 to 41). Unlike the control animals, the number of significant plasma LH concentrations was not found to be elevated during days -1 to 2, 7 to 10 or 11 to 14. Fluctuations in plasma FSH and PRL concentration in the ovariectomized animals were also distributed uniformly throughout the 20-day collection interval (tables 39 to 41).

4. Discussion. Many studies have been published which document the stimulatory effect of ovariectomy on both pituitary and plasma gonadotropin concentrations. A decline in plasma steroid concentration decreases the hypothalamic inhibition of gonadotropin releasing hormone (GnRH) thus increasing the concentration of this hormone in the portal circulation of the anterior pituitary gland where GnRH stimulates gonadotropin secretion (McCann et al., 1965; Schally et al., 1967; Matsuo et al., 1971). In the pig, continual absence of steroid feedback has been shown to increase both pituitary and plasma gonadotropin concentrations (Parlow et al., 1964; Rayford et al., 1971; Rayford et al., 1973). The data from this study (tables 28 and 29) have also shown that baseline LH and FSH concentrations, in 6 ovariectomized swine, to be significantly greater ($P<.05$) than the controls.

The mean plasma concentrations of porcine LH and FSH were found to be significantly elevated within 10 hrs after ovariectomy. A similar increase in serum gonadotropin concentration was also observed to occur within 24 hrs after ovariectomy in hamsters (Goldman and Porter, 1970) and cattle (Hobson and Hansel, 1972). This precipitous increase in plasma gonadotropin concentration may be due to the reduction of steroidal hypothalamic inhibition of LH and FSH secretion resulting from the decline in the plasma concentrations of gonadal steroids following ovariectomy.

Baseline PRL concentration was not affected as a result of ovariectomy. The reciprocal behavior of PRL in relation to LH and FSH is in keeping with the known physiological mechanisms governing the secretion of these hormones (Wurtman, 1971).

In experiments where blood was collected at frequent intervals (every 15 min) circulating plasma LH concentrations in ovariectomized rats,

sheep and monkeys were shown to fluctuate rhythmically. This cyclic pattern of LH occurs at intervals of 15 to 25 min in the rat (Gay, 1970), 52 min in sheep (Reeves et al., 1972a) and 75 min in monkeys (Dierschke et al., 1970). The pulsatile discharge of hormone does not appear to be peculiar to LH release in ovariectomized animals since a similar phenomenon has also been reported in intact women (Kapen et al., 1973). The LH and FSH peaks described in this study are probably due to this pulsatile discharge of LH by the porcine pituitary. Dierschke et al. (1970) hypothesized that LH release was primarily controlled by the central nervous system and in the intact animal, steroid feedback to the hypothalmus served to modulate this rhythmic hormone release. The increased variance in the LH and FSH baseline hormone concentrations appears to support this hypothesis (tables 31 and 32).

The analysis of LH concentrations at the 0600, 1200, 1800 and 2400 hr collection periods revealed that plasma LH concentration was highest at 2400 and 0600 hrs and lowest at 1200 and 1800 hrs. This pattern was identical to that observed in the control and sham treated animals. The consistent daily fluctuation of plasma LH concentration does not appear to be dependent on steroid feedback since the mechanism governing this phenomenon was unaffected by ovariectomy. A change in the metabolic clearance rate of LH also seems doubtful, since plasma FSH and PRL concentrations are not altered.

Considerable variability was observed between animals in regard to the relative rate of increase in gonadotropin concentration after ovariectomy. Yen and Tsai (1971) have observed that the rate of increase in plasma gonadotropin concentration varies in relation to the stage of the cycle in which ovariectomy was performed. These investigators hypothesized that the increased release of LH and FSH in animals ovariectomized

during the follicular phase of the cycle resulted from the stimulatory preconditioning effects of estrogen on gonadotropin synthesis. Since ovariectomy was performed during days 3 to 10, in this study, some of the variability in gonadotropin concentration in response to ovariectomy may be due to differences in the pituitary's ability to synthesize hormone.

G. Analysis of Plasma LH, FSH and PRL Concentrations in Hysterectomized Swine

1. Introduction. The early experiments of Loeb (1923) demonstrated that the uterus of the guinea pig was capable of interacting with the ovary to shorten the functional lifespan of the corpus luteum. Loeb (1923) observed that the lifespan of the corpus luteum was lengthened following hysterectomy. He proposed that the uterus produced a substance which shortened the functional lifespan of the corpora lutea during the non-fertile estrous cycle. This substance has become known as the hypothetical "luteolysin" or "luteolytic factor". Hysterectomy has since been shown to have a similar effect in a number of other species (Caldwell, 1970).

The regression of the corpora lutea during the estrous cycle of the unmated pig has also been shown to be dependent upon a uterine stimulus (Anderson et al., 1961). When gilts were hysterectomized before day 16 of the estrous cycle, the functional lifespan of the corpora lutea was prolonged for approximately 120 days. These investigators found that either the presence of the conceptus or the absence of the uterus (hysterectomy) will inhibit this luteolytic stimulus.

Belt et al. (1970) demonstrated that corpora lutea which were maintained as a result of hysterectomy were histologically similar to those maintained during pregnancy. Masuda et al. (1967) also demonstrated a similarity in progesterone content in the blood and luteal tissue of hysterectomized and pregnant swine. In addition to the functional and histological similarities between corpora lutea from hysterectomized and pregnant gilts, hypophysial support is required to maintain these tissues beyond day 16 of the estrous cycle (Sammelwitz and Nalbandov, 1958; Sammelwitz et al., 1961; du Mesnil du Buisson, 1964; Anderson et al.,

1967).

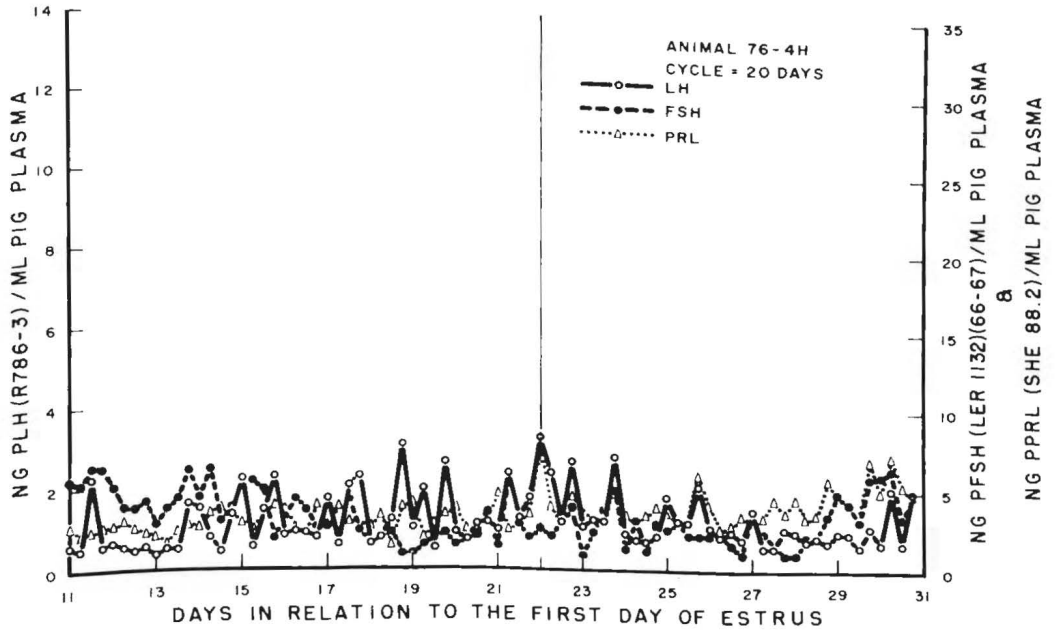
Some workers have proposed that the pituitary may play a role in the process of luteal regression by withdrawing the support of a luteotropin at some critical time during the estrous cycle or by releasing a luteolysin. In order to examine this hypothesis, blood samples were collected during the first 24 days after hysterectomy and plasma LH, FSH and PRL concentrations were analyzed and compared to hormone concentrations in the control and sham operated swine.

2. Materials and Methods. Six Yorkshire sows were hysterectomized between days 5 and 7 of the estrous cycle. An indwelling cannula was inserted into the vena cava of each animal and blood samples were collected at 6 hr intervals (beginning at 0600 hrs) over a period of 24 days. Plasma was removed by centrifugation and stored until assayed by radioimmunoassay. However, blood samples collected during the first 3 days of cannulation were not included in the data analysis. Six unoperated and 6 laparotomized swine described under sub-heading C, D and E of section IV., Results were used as controls. The animals were housed in special holding stalls in the Large Animal Laboratory at the University of Maryland so that temperature and light could be controlled.

The mean of the basal population of hormone concentrations was computed with the truncation procedure (sub-heading B of section IV., Results). All hormone fluctuations were evaluated in relation to baseline. Standard factorial analysis of variance was used to evaluate the effect of treatment on plasma LH, FSH and PRL concentrations.

3. Results. The temporal pattern of plasma LH, FSH and PRL concentrations in representative control, sham and hysterectomized swine are shown in figures 17, 18 and 21. Baseline LH, FSH and PRL concentrations in the hysterectomized animals were not significantly different from the

Figure 21. The temporal pattern of plasma LH, FSH and PRL concentrations between days 4 to 24 after hysterectomy in animal 76-4H. All data collections begin at 0600 hrs on the third day of cannulation.


ANIMAL 76-4H
CYCLE = 20 DAYS
LH
FSH
PRL
NG PLH (R786-3) / ML PIG PLASMA
NG PFSH (LER 1132)(66-67)/ML PIG PLASMA
&
NG PPRL (SHE 88.2)/ML PIG PLASMA
DAYS IN RELATION TO THE FIRST DAY OF ESTRUS
0
2
4
6
8
10
12
14
0
5
10
15
20
25
30
35
11
13
15
17
19
21
23
25
27
29
31

baseline concentrations in the control and sham operated animals ($P>.05$, tables 28 to 30). In addition, the variance (standard deviation) associated with the basal LH, FSH and PRL hormone concentrations was not affected by hysterectomy (tables 31 and 32). The number of LH and FSH plasma concentrations which were found to be significantly different from baseline in the hysterectomized animals was not significantly different ($P>.05$) from the number of LH and FSH concentrations excluded from the mean of the basal population in the control and sham treated swine (tables 33 and 34). However, significantly fewer ($P<.05$) PRL concentrations were found to be significantly different from baseline in the hysterectomized animals than were excluded from the mean of the basal population in the control and sham treated animals (tables 33 and 34).

The temporal fluctuation of plasma LH concentrations in the hysterectomized swine was similar in magnitude and duration to the LH fluctuations observed during the luteal phase of the porcine estrous cycle. LH concentrations which were significantly greater than baseline were found to occur at intervals of approximately 37.20±22.27 hrs throughout the 20-day collection period. Seventy-four percent of the LH peaks contained only 1 concentration which was significantly different from baseline (class 1 peaks) while the remaining peaks contained at least 2 LH concentrations which were significantly different from baseline (class 2 peaks). Class 1 LH peaks rose from baseline to a maximum concentration of 2.15± .31 ng LH/ml plasma and returned to baseline concentrations in 12.92±.33 hrs (table 42). The maximum concentration and duration of the class 2 LH peaks were significantly greater than those observed in the class 1 peaks (class 2 2.63±.23 ng/ml *vs* class 1 2.15±.30 ng/ml, $t=3.09^{*}$, $df=10$; class 2 19.50±1.49 hrs *vs* class 1 12.92±.33 hrs, $t=10.56^{**}$, $df=10$, table 42). The class 1 and class 2 peaks which were observed in the hysterectomized swine

occurred approximately 12.69±1.08 and 12.15±2.54 days after hysterectomy and 20.47±.94 and 19.93±2.37 days after the last observed estrus.

The concentration of plasma LH associated with the class 1 and class 2 peaks was significantly less than the plasma LH concentrations observed during the preovulatory LH surge in the control and sham (control + sham) treated swine (PrOvLH 7.06±2.40 ng/ml vs class 1 2.15±.30 ng/ml, t=4.90**, df=16; PrOvLH 7.06±2.40 ng/ml vs class 2 2.63±.23 ng/ml, t=4.43**, df=16, tables 14 and 42). The duration of the preovulatory LH peak in the control and sham treated swine was also significantly greater than that observed for either class 1 or class 2 peaks (PrOvLH 28.75±4.76 hrs vs class 1 12.92±.33 hrs, t=8.01**, df=16; PrOvLH 28.75±4.76 hrs vs class 2 19.50±1.49 hrs, t= 4.59**, df=16, tables 14 and 42). The magnitude and duration of the class 1 luteal phase peaks in the control and sham treated animals were not significantly different (P>.05) from the magnitude and duration of the class 1 LH peaks in the hysterectomized swine (control + sham class 1 2.29±.55 ng/ml vs Hyst class 1 2.15±.31 ng/ml, t=.54, df=16; control + sham class 1 12.65±1.79 hrs vs Hyst class 1 12.92±.33 hrs, t=.37, df=16). The magnitude and duration of the class 2 LH peaks in the control animals were not significantly different (P>.05) from the magnitude and duration of the class 2 peaks in the hysterectomized animals (control + sham class 2 2.86 ±1.04 ng/ml vs Hyst class 2 2.63±.23 ng/ml, t=1.43, df=16; control + sham class 2 18.61±3.49 hrs vs Hyst class 1 19.50±1.49 hrs, t=.58, df=16, tables 14 and 42).

Plasma FSH peaks (2.0±1.22 peaks/20-day collection period) in the hysterectomized znimals were scattered throughout the 20-day collection period. The average interval from the day of surgery to the FSH peak was only 8.24±3.32 days (16.08±3.03 days after the last observed estrus). These FSH peaks rose from baseline to a maximum concentration of 8.12±

1.95 ng FSH/ml plasma and returned to baseline in 21.00±4.51 hrs (table 42).

The magnitude of the FSH peaks in the control and sham treated animals was not significantly different (P>.05) from the maximum concentration observed in the hysterectomized animals (PoOvFSH 13.14±9.42 ng/ml vs Hyst FSH 8.12±1.95 ng/ml, t=1.16, df=12; $\bar{x}$MCFSH 9.76±3.78 ng/ml vs Hyst FSH 8.12± 1.95 ng/ml, t=.88, df=10, tables 18 and 42). The duration of the postovulatory FSH peak was significantly greater than that observed for the hysterectomized FSH peak (PoOvFSH 58.17±16.25 hrs vs Hyst 21.00±4.51 hrs, t= 4.93**, df=12). However, the duration of the average mid-cycle FSH peak was not significantly different from the FSH peak observed in the hysterectomized animals ($\bar{x}$MCFSH 18.09±5.36 hrs vs Hyst FSH 21.00±4.51 hrs, t=1.04, df=10, tables 18 and 42).

Baseline PRL concentrations in the hysterectomized animals were not significantly different from those observed in the control and sham treated animals; however, only 4.17±2.40 prolactin concentrations were found to be significantly different from the baseline in the hysterectomized swine. These significant PRL concentrations occurred thourghout the 20-day collection period (15.28±4.79 days, average interval from day of surgery to PRL peak; 23.06±4.89 days, average interval from last estrus to day of PRL peak). Plasma PRL concentration in the hysterectomy PRL peaks rose from baseline to a maximum concentration of 10.18±2.95 ng PRL/ml plasma and returned to baseline in 17.69±3.50 hrs (table 42). The magnitude and duration of the PrOvPRL and FoPRL peaks in the control and sham treated animals were not significantly different from the magnitude and duration of the hysterectomy PRL peaks (PrOvPRL 11.49±2.97 ng/ml vs Hyst PRL 10.18±2.95 ng/ml, t=.89, df=16, PrOvPRL 20.54±4.02 hrs vs Hyst PRL 17.69±3.50 hrs, t=1.48, df=16, FoPRL 14.28±4.27 ng/ml vs Hyst PRL 10.18±2.95 ng/ml, t=2.10, df=16; FoPRL 18.98±3.30 hrs vs Hyst PRL 17.69±3.50 hrs, t=.77, df=16, tables 24 and 42).

An analysis of plasma hormone concentrations at the 0600, 1200, 1800

and 2400 hr collection periods indicated that LH concentrations at 0600 and 2400 hrs were significantly greater ($P<.05$) than the LH concentrations at 1200 and 1800 hrs (tables 43 and 44). The daily fluctuations of plasma LH concentration in hysterectomized swine were similar to the previously observed pattern of LH in the control, sham and ovariectomized animals. Although the analysis of plasma PRL concentrations at the 4 collection periods failed to indicate a significant effect of treatment in the control, sham or ovariectomized swine, significant differences were noted in the hysterectomized swine. Plasma PRL concentration at 2400 hrs was significantly greater ($P<.05$) than the plasma concentration at 1800 hrs. An analysis of plasma FSH concentrations indicated no significant differences ($P>.05$) in hormone concentration during the 4 collection periods (tables 43 and 44).

Hysterectomy in the nonpregnant pig induces a dramatic change in the temporal profiles of plasma gonadotropin concentrations (tables 38 to 41). The plasma LH concentrations which were found to be significantly different from baseline in the hysterectomized swine (days 7 to 14) were similar in number, magnitude and duration to the LH concentrations which were excluded from baseline in the control and sham treated swine during this same period (table 38). However, a significantly greater number of LH concentrations was observed between days 15 to -2 in the hysterectomized swine (control + sham 15 to -2, 2.08±1.68 conc>BL vs Hyst 15 to -2, 4.67± 1.21 conc>BL, $t=3.34^{**}$, df=16, tables 38 and 41).

The number of LH concentrations which were significantly different from baseline in the hysterectomized animals during days -1 to 2 was not significantly different from the number of concentrations excluded from baseline in the control and sham operated animals (control + sham -1 to 2, 5.92±2.02 conc>BL vs Hyst -1 to 2, 5.67±1.97 conc>BL, $t=.25$, df=16).

Although the number of significant LH concentrations which were excluded during days -1 to 2 was not significantly different, the significant LH concentrations in the control and sham treated swine were part of 1 large LH peak (preovulatory LH peak) whose magnitude and duration were significantly larger than those observed for the significant LH peaks in the hysterectomized animals. The small elevations in plasma LH concentration during days 15 to -2 in the hysterectomized animals were similar in magnitude and duration to the mid-cycle LH peaks observed in the control animals. These hysterectomy LH peaks were found to occur in large numbers during days -1 to 2 (12 to 16 days after hysterectomy) and then decline in frequency during the remainder of the collection interval.

The distribution of significant FSH concentrations during the 20-day collection period was not affected by hysterectomy (tables 39 and 41). However, the temporal profile of significant FSH concentrations during days 3 to 6 in the hysterectomized swine was markedly different from the distribution of significant plasma FSH concentrations in the control and sham treated animals during this same period (control + sham 3 to 6, 2.5±2.07 conc>BL vs Hyst 3 to 6, 0.0±0.0 conc>BL, t=2.65*, df=13, table 39).

Significant plasma PRL concentrations were evenly distributed throughout the 5 four-day collection intervals in hysterectomized swine (tables 40 and 41). The uniform distribution of significant PRL concentrations in hysterectomized animals was significantly different from the distribution of significant PRL concentrations in the control and sham treated animals during days 15 to -2 and -1 to 2 (control + sham 15 to -2, 5.58±2.54 conc>BL vs Hyst 15 to -2, .33±.52 conc>BL, t=4.94**, df=16; control + sham -1 to 2, 4.91 ±3.65 conc>BL vs Hyst -1 to 2, .5±.84 conc>BL, t=2.88**, df=16, table 40).

4. Discussion. Comparative studies of the corpus luteum in hysterectomized and pregnant pigs have revealed numerous structural and

functional similarities (Belt et al., 1970; Masuda et al., 1967). It has also been established that hypophysial support is required to maintain the corpora lutea of pregnant or hysterectomized swine beyond day 16 (Sammelwitz and Nalbandov, 1958; Sammelwitz et al., 1961; du Mesnil du Buisson and Leglise, 1963; Anderson et al., 1967). The structural and functional similarities as well as the hormonal requirements of the corpora lutea in pregnant and hysterectomized pigs suggests that similar physiological mechanisms are involved in the maintenance of the corpora lutea in both of these physiological states. Hysterectomized animals were used as a model to examine plasma hormone concentrations under induced physiological conditions which are known to maintain the porcine corpus luteum.

The first direct evidence of the involvement of the pituitary in the maintenance of the corpus luteum in hysterectomized swine was presented by du Mesnil du Buisson and Leglise (1963). These investigators showed that corpora lutea in hysterectomized swine regressed 5 to 12 days after hypophysectomy. After initiating replacement therapy, du Mesnil du Buisson (1964) observed that LH preparations maintained corpora lutea to the twentieth day in hypophysectomized-hysterectomized swine, but they could not be maintained beyond this point. These preliminary observations were confirmed by Anderson et al. (1967). The administration of LH or HCG into the hypophysectomized-hysterectomized animals could also maintain the secretory function of the corpora lutea while PRL alone could not. It was emphasized that none of these pituitary hormones could maintain the corpora lutea in the presence of the uterus. The induced regression of the corpora lutea with anti-LH antiserum also supports the hypothesis that LH is luteotropic in the pregnant pig (Spies et al., 1967).

The analysis of plasma LH concentrations in hysterectomized swine

during days 10 to 30 has clearly demonstrated an increase in plasma LH concentration between days 18 to 25 (figure 21, table 38). Although pituitary LH concentrations have been shown to be elevated during days 18 and 25 in both pregnant and hysterectomized swine (Melampy et al., 1966; Parlow et al., 1964) the data presented in this section are the first to demonstrate a significant elevation in plasma LH concentration at a time when pituitary support is known to be required for luteal maintenance. The absence of a consistent pattern of significant FSH and PRL concentrations during the 20-day collection period suggests that these hormones are not of primary importance in the maintenance of the porcine corpus luteum.

Some workers have proposed that the pituitary may play a role in the process of luteal regression. Caldwell (1970) proposed that the pituitary may withdraw the support of a luteotropin at some critical time during the estrous cycle, or may even release a luteolysin. The plasma hormone concentrations obtained in this study tentatively support this hypothesis. Evidence presented in this study and in others strongly supports the hypothesis that LH is luteotropic in the pig. An increase in plasma estrogen concentration during the follicular phase of the porcine estrous cycle is presumably responsible for the sub-basal LH concentrations observed in the control and sham treated animals (Henricks et al., 1972). The presumed inhibitory effect of follicular phase steroids during days 16 to -1 may serve to suppress plasma LH concentrations, thus preventing the luteotropic effect of LH in the non-gravid pig.

The studies of Henricks et al. (1972) and Guthrie et al. (1972) have demonstrated that plasma estrogen concentrations begin to rise prior to or coincident with the regression of the corpus luteum in the pig. Decreasing progesterone concentrations in conjunction with rising estrogen

concentrations, were hypothesized as the stimulus for the follicular phase prolactin peaks observed in the control and sham operated animals (section E). The elevation of plasma PRL concentration, late in the luteal phase of the estrous cycle, merits further investigation since PRL has been shown to be luteolytic in the rat under similar conditions (Malven and Sawyer, 1966; Wuttke and Meites, 1971). The absence of elevated PRL concentrations during days 16 and 17 in the hysterectomized swine is noteworthy since the absence of this hormone, at a time when the corpora lutea are being maintained, further supports the hypothesis that the follicular phase PRL peak may be luteolytic in the intact pig.

The elevation of plasma LH concentration during the night hrs is consistent with the nocturnal LH pattern previously observed in the control, sham and ovariectomized animals. The consistency of this pattern, irrespective of the physiological treatment, suggests that these nocturnal elevations are due to some basic physiological mechanism which governs the secretion or metabolism of this hormone. A similar nocturnal increase in plasma PRL concentration was also noted in the hysterectomized animals. The significance of this observation must await further experimentation since the majority of the PRL concentrations in the hysterectomized swine were not significantly different from baseline.

V. GENERAL DISCUSSION

The experimental design utilized in this study has provided data on both the daily and long-term temporal profiles of plasma LH, FSH and PRL concentrations without adversly affecting the physiological state of the animals. The removal of 10 ml of blood at 6 hr intervals over a period of 20 days did not significantly alter red and white blood cell number or hematocrit counts. The stability of PRL concentrations during the 20-day collection period also supports the hypothesis that the animals were not stressed during their confinement. Controlled environmental condition in conjunction with the ability to remove blood samples any time of the day or night without adversly affecting the animals, has provided a means by which hormone fluctuation can be studied in the "normal" pig.

The large number of observations collected during this study presented a formidable problem since the application of statistical theory to the analysis of such data had not yet found its way into the literature. A possible reason for the absence of statistics in the analysis of estrous cycle data may be due to the lack of an acceptable starting point. For example, in order to establish the relative importance of the preovulatory LH surge in statistical terms, there must exist some basis of comparison between that event and other events occurring within the same estrous cycle. The most meaningful choice upon which to base amplitude comparisons would be baseline, since it is the point from which all other deviations occur.

Although numerous procedures could be used to obtain estimates of baseline, the technique used in this study has several distinct advantages:

1. The criteria utilized in the estimation of baseline were estab-

lished before the data were collected; thus, preventing the investigator from biasing his baseline estimate by preferentially selecting specific data points.

2. Baseline is represented as a mean, making it possible to obtain an estimate of the sample variance about the mean.

3. A confidence envelope may be established around the baseline, making it possible to statistically evaluate points which deviate from the baseline.

4. The basic theory utilized in deriving a statistic which is representative of baseline can be applied to different treatments (e. g., evaluation of hormone concentrations obtained during the normal estrous cycle, ovariectomy or hysterectomy).

5. The procedure can be readily computerized, expiditing the analysis of the data.

The collection of plasma samples at 6 hr intervals and their subsequent statistical analysis has permited an objective appraisal of the temporal profile of plasma hormone concentrations during different physiological states.

Previous studies of hormone concentrations in the peripheral plasma of the pig have been routinely limited to 1 observation per day except during estrus, where more frequent observations have been reported. The lack of information relating to endogenous hormone concentrations during the remainder of the porcine estrous cycle has prevented a thorough understanding of the mechanisms which govern the porcine estrous cycle. An analysis of plasma hormone concentrations indicated that LH concentrations associated with the preovulatory surge routinely peaked during the night hrs and reached magnitudes which were generally greater than those previously reported in the literature (Niswender et al., 1970; Tillson et al., 1970; Rayford

et al., 1971; Henricks et al., 1972). Sampling the blood of the pig at 6 hr intervals, during the luteal phase of the estrous cycle, revealed the presence of LH concentrations which were found to be significantly different from baseline. These luteal phase peaks occurred between days 4 to 14 with the maximum number of peaks occurring on day 9. After the corpora lutea had regressed, LH concentrations were no longer found to be significantly different from baseline.

These data indicate that luteal phase progesterone concentrations are not capable of completely inhibiting LH secretion. The numerous significant LH concentrations observed in the hysterectomized animals also support the hypothesis that progesterone cannot completely inhibit the secretion of LH. Previous studies of the mechanisms governing LH secretion in ovariectomized animals have revealed a pulsatile pattern of LH release (Gay et al., 1970; Dierschke et al., 1970; Butler et al., 1971; Reeves et al., 1972a). It has been hypothesized that the cyclic release of gonadotropins in the ovariectomized animals is controlled by the central nervous system (Dierschke et al., 1970). If a similar mechanism controls LH release in the intact pig, LH secretion may be controlled by intermittent signals from the central nervous system which in turn serve to modulate the synthesis and secretion of LH releasing factor and the eventual discharge of LH.

Steroid feedback is known to alter both the levels of neurotransmitter in the central nervous system and the enzymes which control catecholamine metabolism (Kurachi et al., 1968; Wurtman, 1971). Wurtman (1971) has suggested that the nocturnal fluctuation of LH in men may be caused by fluctuations in catecholamine concentration during rapid eye movement sleep. Since 75 percent of the luteal phase LH peaks occur during the night hrs, nocturnal LH fluctuations in the pig may also be controlled by central

nervous system neurotransmitters.

The mechanisms responsible for the luteal phase LH peaks may be academic if these small plasma LH fluctuations do not exert a significant biological effect. During certain physiological states, small changes in plasma LH concentration would appear to have biological importance since small plasma LH fluctuations have been observed in the hysterectomized pig at a time when hypothalamic support is known to be required for luteal maintenance. The physiological significance of the luteal phase LH fluctuations is unknown since formation of the porcine corpora lutea during the luteal phase of the estrous cycle has been shown to occur independent of hypophysial support (Sammelwitz et al., 1961; Brinkley et al., 1964a,b; du Mesnil du Buisson and Leglise, 1963). The data presented by these authors is consistent with the hypothesis that luteotropic support is not required for "structural maintenance". However, the independence of luteotropic support in regard to the secretory function of the corpus luteum has not been fully established. Brinkley et al. (1964b) found progesterone content to be reduced in response to progesterone administration. The total amount of progesterone per treatment was also significantly lower ($P<.05$) than that of the controls when progesterone treatment was initiated on days 1 or 2. Spies et al. (1967) demonstrated that the administration of 1 ml of anti-LH antiserum during days 7 through 17 of the estrous cycle is capable of reducing progesterone content by 40 percent. Both in vivo and in vitro studies have shown that LH is capable of stimulating the synthesis and secretion of progesterone (Cook et al., 1969; Channing, 1970; Van Thiel et al., 1971). The significance of the mid-cycle LH peaks must await further experimentation, although the observation of structural and functional changes in the corpus luteum during the middle of the luteal phase of the estrous

cycle (Cavazos et al., 1969; Caldwell et al., 1969) and the occurrence of waves of follicular growth and atresia (Green, 1950) are consistent with the hypothesis that elevated luteal phase LH concentrations may be involved in the control of the estrous cycle.

The greatest increase in plasma FSH concentration occurs between days 2 and 3 of the estrous cycle. This increase in plasma FSH concentration occurs at a time when follicle size is less than 4 mm (Green, 1950) and both progesterone and estrogen concentrations are known to be low (Henricks et al., 1972). The absence of steroid feedback presumably initiates the release of FSH and the ensuing rapid follicular development observed between days 3 to 9 (Green, 1950; Parlow et al., 1964). Since FSH concentration remains at intermediate concentrations during the remainder of the luteal phase of the estrous cycle, FSH may also be involved with the growth and atresia of luteal phase follicles.

The most perplexing observation in regard to the temporal profile of plasma FSH concentration is the absence of an FSH peak during the follicular phase of the estrous cycle. The rapid follicular growth observed during the follicular phase of the estrous cycle has been routinely attributed to FSH stimulation. Sub-basal concentrations of plasma FSH and LH during the follicular phase of the estrous cycle suggest that the large ovarian follicles do not require elevated gonadotropin concentration in order to "mature". A decrease in gonadotropin support during the follicular phase should inhibit follicular growth. An extensive study of ovarian morphology by Green (1950) supports this hypothesis since the total number of follicles were found to decrease as the "chosen follicles" increased in size. The ability of the large follicles to survive under these conditions may be due to an increase in the number of hormone receptor sites. Studies relating the number of LH receptor sites to fol-

licle size support this conclusion (Channing and Kammerman, 1973).

The importance of prolactin in the control of the porcine estrous cycle has not been established. Studies with hypophysectomized-hysterectomized swine suggest that prolactin is not luteotropic in the pig (Anderson et al., 1967). This conclusion is also supported by the prolactin data from this study. The consistency and magnitude of the follicular phase prolactin peak during the normal estrous cycle suggests that prolactin may be involved with the regression of the corpora lutea. The luteolytic effects of prolactin in the rat (Wuttke and Meites, 1971) supports this conclusion. Additional experiments are needed to investigate this hypothesis.

Ovariectomy in the pig elevates plasma LH and FSH concentrations while prolactin concentrations were unchanged. These observations are in keeping with the theories of steroid feedback inhibition. An elevation in plasma gonadotropin concentration was found to occur within hrs after removal of the ovaries. This rapid rise in plasma gonadotropin concentration in response to ovariectomy is thought to be correlated with the metabolic clearance of endogenous ovarian steroids (Goldman and Porter, 1970).

The large number of plasma LH concentrations which were found to be significantly different from baseline in the hysterectomized pig suggests that LH is the gonadotropin responsible for maintaining the corpora lutea in hysterectomized swine. The luteotropic effects of LH in the hysterectomized pig were previously described by Anderson et al. (1967). However, the data presented in this study are the first to demonstrate a significant elevation in endogenous plasma LH concentration at a time when pituitary support is known to be required for luteal maintenance. The absence of a consistent pattern of significant plasma FSH and PRL concentration suggests that these hormones do not play a major role in main-

taining the corpora lutea in the hysterectomized pig.

VI. SUMMARY

A critical assessment of plasma hormone concentrations is an essential prerequisite to a basic understanding of the physiological mechanisms governing the reproductive process. Previous studies documenting the preovulatory LH surge in the pig, have indicated that minor fluctuations in gonadotropin concentrations are capable of initiating major physiological events. The low concentration of gonadotropin required to induce a physiological change, in conjunction with an overall lack of understanding in regard to the pattern of gonadotropin concentrations in the pig, necessitated a critical study of plasma hormone concentrations during the estrous cycle. The descriptive data provided by this study will enable future investigators to design critical experiments to evaluate the physiological importance of these hormonal fluctuations thereby broadening our basic understanding of the mechanisms which govern the reproductive process.

Table 1

HORMONE CONTENT DURING THE PORCINE ESTROUS CYCLE

Source	Hormone	Estrous Cycle (Days) 2-7	8-15	16-20	21-1
Anterior Pituitary Content	LH	LR†	IR	H	L
	FSH	LR	IR	H	L
	PRL	LR	IR	H	L
Plasma Concentrations	LH	L	L	L	H
	FSH	--	--	--	--
	PRL	--	--	--	--
	Prog.Δ	LR	H	HD	L
	Est.	LF	L	LR	HD

† Abbreviations used to describe hormone concentrations during the porcine estrous cycle: LR, low and rising; IR, intermediate and rising; H, high; L, low; HD, high and decreasing; LF, low and fluctuating; --, no available data.

Δ Abbreviations: Prog., progesterone; Est., estrogen.

Table 2

CHARACTERISTICS OF THE RADIOIMMUNOASSAY SYSTEMS†

Assay Parameters	Porcine RIA		
	LH	FSH	PRL
Blank (%)	4.05± .82	4.34± .61	8.85±1.16
Initial Binding (%)	31.93±4.97	19.05±3.35	21.97±4.24
50% Inhibition Point (ng)	.69± .09	10.89±2.23	1.39± .29
Within-Assay Variation (%)	3.04±1.03	2.31± .61	2.38± .60
Between-Assay Variation (%)	11.68	15.64	29.18

† All data were expressed as mean ±1 SD. The porcine LH, FSH and PRL data were based on 24 separate radioimmunoassays except for the between-assay variation of FSH which was based on 5 assays. Blank (0% bound) refers to the fraction of total counts "precipitated" in the absence of specific antiserum (first antibody). The blank counts were subtracted from total (CPM of radioactive hormone added to each assay tube) and bound counts (CPM of radioactive hormone which had been precipitated) for calculation of all radioimmunoassay data. Initial binding is the fraction of total counts bound in the absence of added standard hormone. The 50% inhibition point is the amount of hormone required to effect 50% reduction from the initial binding. Within-assay variation was computed from the mean coefficient of variation of 72 separate unknowns from each assay. Between-assay variation was based on the coefficient of variation of the internal serum standards run on each of the 24 assays.

Table 3

TEST OF THE HYPOTHESIS SLOPE=0 FOR THE REGRESSED PERCENTAGE OF PACKED CELLS VS TIME OF SAMPLE COLLECTION IN HOURS

Animal	df	Slope†	Intercept△	r	t
1-2	20	-.0022	35.94	-.1581	.716
3-3	20	.0044	30.47	.2305	1.035
3-4	20	.0027	31.85	.2421	1.112
4-3	18	.0221	26.31	.7781	5.260**
90-6	19	.0074	29.43	.4339	2.099*
3-6	20	.0022	31.34	.1704	.773
7-1	20	.0011	32.93	.1074	.483
90-5	16	.0104	27.13	.4483	2.006
63-6	18	-.0024	34.33	-.1374	.588
63-9	19	.0146	30.65	.8074	5.967**
63-11	16	-.0037	31.88	-.1978	.807
54-7	19	.0085	29.19	.5643	2.980**
63-10	18	-.0041	34.38	-.5363	2.696**
48-6	19	.0151	28.91	.7418	4.822**
54-6	19	.0072	29.10	.7511	4.959**
58-12	19	.0166	27.96	.7421	4.826**
76-5	20	-.0014	34.43	-.1227	.553
42-10	20	.0025	28.32	.1981	.904
47-10	20	.0068	32.66	.4311	2.137**
76-4	20	.0066	29.05	.5065	2.627*
73-6	20	-.0106	36.58	-.6367	3.692**
48-5	19	-.0009	31.14	-.0631	.276
63-14	19	.0026	32.22	.1701	.712
65-6	20	.0077	38.01	.5034	2.605**

* $P < .05$.

** $P < .01$.

† Expressed as percent packed cells/day.

△ Expressed as percent packed cells at Y-intercept.

Table 4

THE EFFECT OF SURGICAL TREATMENT ON THE PERCENTAGE OF PACKED CELLS ON THE FIRST, TWELFTH AND TWENTY-SECOND DAY OF BLOOD COLLECTION

Treatment	Days		
	1†	12	22
Control	34.30[a]	36.20[a]	34.00[a]
Sham	32.00[a,b,c]	33.30[b]	32.70[a]
Ovariectomized	34.30[a,b]	34.00[a,b]	36.30[a]
Hysterectomized	27.60[c]	33.40[b]	34.50[a]

† Means with same superscript letter are not significantly different ($P>.05$).

Table 5

THE EFFECT OF SURGICAL TREATMENT ON THE PERCENTAGE OF PACKED CELLS ON THE FIRST, TWELFTH AND TWENTY-SECOND DAY OF BLOOD COLLECTION

Analysis of Variance Table

Day	Source	df	SS	MS	F
	Block	5	47.80	8.60	1.25
1	Time of Day	3	177.80	59.30	8.69**
	Error	15	102.30	6.80	
	Block	5	8.90	1.80	.64
12	Time of Day	3	33.20	11.10	4.03*
	Error	15	41.30	2.70	
	Block	5	34.10	6.80	.76
22	Time of Day	3	44.10	13.70	1.54
	Error	14	124.90	8.90	

* $P<.05$.

** $P<.01$.

Table 6

THE EFFECT OF SURGICAL TREATMENT ON THE DAILY PERCENTAGE OF PACKED CELLS DURING DIFFERENT DAYS OF SAMPLE COLLECTION

Treatment	Day of Exp. Period				
	1	8	15	22	Mean†
Control	34.30	34.40	34.80	34.00	34.40[a]
Sham	32.00	32.20	33.10	32.70	32.50[a]
Ovariectomized	34.30	33.60	34.60	36.30	34.70[a]
Hysterectomized	27.60	31.80	33.90	34.50	32.00[a]

† Means with same superscript letter were not significantly different (P>.05).

ANALYSIS OF VARIANCE TABLE

Source	df	SS	MS	F	F'
Blocks	5	26.90	5.38	1.04	
Surgery	3	142.52	47.51	9.17**	3.73*
Day	3	88.87	29.62	5.72**	2.32
Surg. x Day	9	114.66	12.74	2.46*	
Error	78	404.03	5.18		

* P<.05.

** P<.01.

Table 7

THE EFFECT OF TIME OF DAY AND SURGICAL TREATMENT ON THE PERCENTAGE OF PACKED CELLS DURING TWENTY-TWO DAYS OF BLOOD COLLECTION

	Time of Day in Hours				
Treatment	6	12	18	24	Mean†
Control	34.40	36.20	35.50	33.60	34.90[a]
Sham	32.30	33.50	33.30	31.80	32.70[a,b]
Ovariectomized	33.40	35.40	35.10	32.50	34.10[a,b]
Hysterectomized	31.90	33.40	32.30	31.30	32.20[b]
Mean†	33.00[a,b]	34.60[a]	34.10[a,b]	32.30[b]	

† Means with same superscript letter were not significantly different ($P>.05$).

ANALYSIS OF VARIANCE TABLE

Source	df	SS	MS	F
Block	5	26.35	5.27	2.23
Surgery	3	108.92	36.31	15.38**
Time of Day	3	76.36	25.45	10.78**
Surg. x Time of Day	9	6.18	.69	.29
Error	75	177.17	2.36	

** $P<.01$.

Table 8

EFFECT OF SURGICAL TREATMENT ON RED AND WHITE BLOOD CELL NUMBERS IN REPLICATES 5 AND 6 DURING THE MIDDLE AND END OF THE 22-DAY SAMPLE COLLECTION PERIOD

Collection Period	Cells	Control	Sham	Ovar.	Hyst.
Mid	RBC	5,540†	5,250	5,015	5,160
	WBC	18,220Δ	22,380	17,320	17,040
End	RBC	5,100	4,815	5,430	5,130
	WBC	16,880	21,520	22,600	19,180

† Coded Value -- multiply by 1 x 10 to obtain the number of RBC per mm^3.

Δ The number of white blood cells per mm^3.

ANALYSIS OF VARIANCE TABLE

Source	df	SS	MS	F
Blocks	1	8.76	8.76	.102
Surg.	3	21.63	7.21	.084
Period	1	2.80	2.80	.033
Cells	1	1615.96	1615.96	18.770**
Surg. x Period	3	18.07	6.02	.070
Surg. x Cells	3	26.16	8.72	.101
Period x Cells	1	4.08	4.08	.047
Surg. x Period x Cells	3	10.60	3.53	.041
Error	15	1291.42	86.10	

** $P<.01$.

Table 9

SKEWNESS AND KURTOSIS ESTIMATES OF PLASMA LH CONCENTRATIONS IN THE CONTROL, SHAM, OVARIECTOMIZED AND HYSTERECTOMIZED SWINE†

Treatment	Animal	Skewness g_1	Kurtosis g_2	Conc. >BL$^{\Delta}$
Control	1-2	1.86**	2.73**	21
	90-6	2.19**	6.18**	9
	63-6	2.44**	6.71**	21
	63-10	1.69**	3.26**	13
	76-5	1.81**	4.10**	10
	73-6	1.86**	3.88**	8
Sham	3-3	2.35**	6.94**	17
	3-6	3.20**	13.46**	10
	63-9	2.73**	7.08**	19
	48-6	5.07**	33.28**	15
	42-10	4.53**	25.82**	9
	48-5	3.25**	12.71**	15
Ovariectomized	3-4	.04	-.87	0
	7-1	.53*	-.73	5
	63-11	.50*	1.42**	2
	54-6	.62**	-.02	3
	47-10	.46*	-.21	2
	63-14	.17	-.93	0
Hysterectomized	4-3	1.55**	1.79**	16
	90-5	.84**	.84*	5
	54-7	1.49**	1.68**	15
	58-12	1.23**	.82*	26
	76-4	1.05**	.08	21
	65-6	1.15**	.82*	15

† Initial skewness and kurtosis estimates for all plasma hormone concentrations obtained during the 20-day collection period.

Δ Number of plasma hormone concentrations significantly different ($P<.05$) from baseline.

* $P<.05$.

** $P<.01$.

Table 10

SKEWNESS AND KURTOSIS ESTIMATES OF PLASMA FSH CONCENTRATIONS IN THE CONTROL, SHAM, OVARIECTOMIZED AND HYSTERECTOMIZED SWINE†

Treatment	Animal	Skewness g_1	Kurtosis g_2	Conc. >BL$^{\Delta}$
Control	1-2	--	--	--
	90-6	.13	-.62	0
	63-6	.08	-.98	0
	63-10	.84**	.19	6
	76-5	-.45	.02	0
	73-6	.29	-.51	0
Sham	3-3	--	--	--
	3-6	.05	1.12*	0
	63-9	.46*	-.17	2
	48-6	.37	.24	0
	42-10	5.63**	33.62**	3
	48-5	-.01	.01	0
Ovariectomized	3-4	--	--	--
	7-1	-.34	-.95	0
	63-11	-.53	-.14	0
	54-6	-.27	-1.18	0
	47-10	-1.64	4.20**	0
	63-14	-.26	-1.12	0
Hysterectomized	4-3	--	--	--
	90-5	-.49	.30	0
	54-7	-.56	.19	0
	58-12	-.29	-.10	0
	76-4	.46*	-.80	4
	65-6	-.27	-.47	0

† Initial skewness and kurtosis estimates for all plasma hormone concentrations obtained during the 20-day collection period.

$^{\Delta}$ Number of plasma hormone concentrations significantly different ($P<.05$) from baseline.

* $P<.05$.

** $P<.01$.

Table 11

SKEWNESS AND KURTOSIS ESTIMATES OF PLASMA PRL CONCENTRATIONS IN THE CONTROL, SHAM, OVARIECTOMIZED AND HYSTERECTOMIZED SWINE†

Treatment	Animal	Skewness g_1	Kurtosis g_2	Conc. >BL$^{\Delta}$
Control	1-2	2.36**	5.95**	12
	90-6	2.94**	11.40**	7
	63-6	1.65**	2.72**	12
	63-10	.19	.19	0
	76-5	2.07**	3.21**	13
	73-6	2.67**	7.12**	13
Sham	3-3	1.66**	4.44**	4
	3-6	1.28**	1.54**	20
	63-9	.41	.41	0
	48-6	1.36**	2.47**	7
	42-10	2.80**	7.91**	16
	48-5	1.25**	1.98**	7
Ovariectomized	3-4	-.04	-.30	--
	7-1	.36	-.18	--
	63-11	-.30	-.27	--
	54-6	.76**	.47	4
	47-10	3.95**	22.87**	4
	63-14	-.41	-.50	0
Hysterectomized	4-3	.37	-.25	--
	90-5	-.08	-.91	--
	54-7	1.18**	2.83**	4
	58-12	2.41**	10.20**	3
	76-4	1.24**	2.14**	5
	65-6	.15	-.24	0

† Initial skewness and kurtosis estimates for all plasma hormone concentrations obtained during the 20-day collection period.

Δ Number of plasma hormone concentrations significantly different ($P<.05$) from baseline.

* $P<.05$.

** $P<.01$.

Table 12

BASELINE ESTIMATES FOR LH, FSH AND PRL HORMONE CONCENTRATIONS (NG/ML PLASMA) IN 6 CONTROL ANIMALS DURING THE PORCINE ESTROUS CYCLE

		Statistic			
			Sample Population$^{\Delta}$		
Hormone	Animal	Truncated Mean†	Mean	Median	Mode
LH	1-2	1.25	1.68	1.33	1.10
	90-6	1.71	1.96	1.67	1.30
	63-6	.95	1.41	1.05	.90
	63-10	.98	1.30	.99	.90
	76-5	.87	1.03	.89	.70
	73-6	1.29	1.59	1.27	1.30
	Mean	1.18	1.50	1.20	1.03
	±1 SD	.31	1.69	.28	.24
FSH	1-2	--	--	--	--
	90-6	4.00	4.05	3.92	2.70
	63-6	7.73	7.84	7.54	10.50
	63-10	5.83	6.13	5.88	5.30
	76-5	5.40	5.44	5.37	4.50
	73-6	3.94	3.99	3.97	4.30
	Mean	5.38	5.49	5.34	5.46
	±1 SD	1.56	1.60	1.50	2.98
PRL	1-2	5.97	6.64	6.00	5.50
	90-6	7.33	7.96	7.27	6.90
	63-6	7.12	7.56	7.25	6.70
	63-10	5.18	5.22	5.13	5.10
	76-5	5.09	6.45	5.16	4.70
	73-6	6.26	7.89	6.39	6.30
	Mean	6.16	6.95	6.20	5.87
	±1 SD	.94	.97	.95	.90

† Truncated mean - represents the mean of a normalized population of hormone concentrations (e. g., LH, FSH or PRL) obtained by the removal of all concentrations which contributed to a positive skewness estimate.

Δ Sample population - represents all hormone concentrations (e. g., LH, FSH or PRL) collected during one estrous cycle.

Table 13

A SUMMARY OF THE GENERAL BIOLOGICAL DATA RELATING TO PLASMA LH CONCENTRATIONS IN CONTROL AND SHAM OPERATED SWINE

	Control		Sham		Control + Sham			
Variable[Δ]	Mean ± 1 SD		Mean ± 1 SD		Mean ± 1 SD		t[†]	df
Cycle length (days)	19.83	1.33	20.50	.55	20.17	1.03	1.14	10
Baseline (ng/ml)	1.17	.31	1.21	.19	1.19	.25	.31	10
Baseline SD (ng/ml)	.30	.11	.24	.07	.27	.09	1.27	10
Grand Mean[∇] (ng/ml)	1.50	.32	1.52	.16	1.51	.24	.19	10
Grand Mean SD (ng/ml)	.95	.23	1.03	.28	.99	.24	.55	10
Median[∇] (ng/ml)	1.20	.39	1.24	.18	1.22	.23	.30	10
Mode[∇] (ng/ml)	1.03	.24	1.20	.21	1.12	.23	1.27	10
Conc.>BL + 2 SD (conc./ estrous cycle)	16.50	5.68	17.17	4.12	16.80	4.75	.23	10
No. of LPP	8.50	3.54	8.33	2.16	8.75	2.16	.78	10

† The t-test was used to test for significant differences between the control and sham treated swine. A pooled mean and standard deviation were not computed if t was significant at the 5% level.

Δ Abbreviations: BL, baseline; SD, standard deviation; LPP, luteal phase peaks.

∇ Computed from all hormone concentrations (e. g., LH, FSH or PRL) collected during one estrous cycle.

Table 14

THE NUMBER, MAGNITUDE AND DURATION OF LH PEAKS DURING THE ESTROUS CYCLE OF THE CONTROL AND SHAM OPERATED SWINE

	Control		Sham		Control + Sham			
Variable[Δ]	Mean	1 SD	Mean	1 SD	Mean	1 SD	t[†]	df
No. of Peaks								
PrOvLH	1.00	--	1.00	--	1.00	--	--	--
Class 1	5.83	2.56	6.33	2.66	6.08	2.50	.33	10
Class 2	2.67	1.03	2.00	1.67	2.33	1.37	.83	10
Magnitude (ng/ml)								
PrOvLH	6.41	2.12	7.70	2.69	7.06	2.40	.92	10
PrOvLH - BL	5.24	2.16	6.49	2.71	5.86	2.43	.88	10
PrOvLH - (BL+2 SD)	4.64	2.15	6.02	2.70	5.83	2.44	.98	10
Class 1	2.34	.75	2.23	.33	2.29	.55	.33	10
Class 1 - BL	1.21	.56	1.02	.34	1.11	.45	.74	10
Class 2	3.21	1.20	2.34	.47	2.86	1.04	1.36	8
Class 2 - BL	1.96	1.06	1.21	.41	1.66	.91	1.33	8
Duration[∇] (hrs)								
PrOvLH	28.52	6.11	28.98	3.52	28.75	4.76	.16	10
PrOvLH (BL+2 SD)	29.30	10.59	34.33	6.94	31.81	8.93	.97	10
Class 1	11.78	1.76	13.52	1.47	12.65	1.79	1.86	10
Class 1 (BL+2 SD)	4.81	3.00	5.29	1.58	5.05	2.30	.35	10
Class 2	18.30	4.22	19.08	2.53	18.61	3.49	.33	8
Class 2 (BL+2 SD)	12.58	1.45	12.26	3.23	12.45	2.16	.22	8

† The t-test was used to test for significant differences between the control and sham treated swine. A pooled mean and standard deviation were not computed if t was significant at the 5% level.

Δ Abbreviations and definitions: PrOvLH, preovulatory LH peak; BL, baseline; BL + 2 SD, baseline + 2 standard deviations; Class 1, contain 1 concentration which was significantly greater than baseline; Class 2, contain 2 or more concentrations which were significantly greater than baseline.

∇ Duration estimates were computed in relation to baseline and baseline + 2 standard deviations.

Table 15

TIME INTERVAL (DAYS) BETWEEN LH PEAKS WITHIN THE ESTROUS CYCLE OF THE CONTROL AND SHAM OPERATED ANIMALS

Interval† Between Peaks▽		Control		Sham		Control + Sham			
From	To	Mean	± 1 SD	Mean	± 1 SD	Mean	± 1 SD	t△	df
PrOvLH	FMCLH	3.68	2.07	3.14	1.85	3.41	1.89	.47	10
PrOvLH	LMCLH	14.78	1.89	17.16	1.99	15.97	2.23	2.14	10
LMCLH	PrOvLH	5.24	2.22	3.71	1.88	4.48	2.12	1.28	10
PrOvLH	$\bar{x}$ MCLH	9.04	.78	9.94	1.20	9.49	1.07	1.55	10
FMCLH	LMCLH	11.01	2.43	14.09	3.27	12.55	3.19	1.85	10
Mean of consecutive adjacent mid-cycle peaks		1.56	.55	1.99	.69	1.77	.64	1.17	10

▽ Abbreviations of LH peaks: PrOvLH, preovulatory LH; FMCLH, first mid-cycle LH; LMCLH, last mid-cycle LH; $\bar{x}$ MCLH, average mid-cycle LH.

△ The t-test was used to test for significant differences between the control and sham treated swine. A pooled mean and standard deviation were computed if t was significant at the 5% level.

† Peak intervals were computed by measuring the distance between parallel lines drawn perpendicular to baseline from points located in the center of each peak at 1/2 peak height.

Table 16

THE EFFECT OF TIME OF DAY
ON LH CONCENTRATIONS

Treatment (hrs)	LH Conc (ng/ml) Mean† ± 1 SD	
0600	1.60[a,b]	.35
1200	1.32[b]	.31
1800	1.23[b]	.29
2400	1.84[a]	.45

† Means with same superscript letter were not significantly different (P>.05).

ANALYSIS OF VARIANCE TABLE

Source	df	SS	MS	F
Block	5	2.07	.41	12.99**
Time of Day	3	1.40	.47	15.81**
Error	15	.44	.03	

** P<.01.

Table 17

THE DISTRIBUTION OF PLASMA LH CONCENTRATIONS FOUND TO BE SIGNIFICANTLY GREATER THAN BASELINE IN RELATION TO THE DAY OF THE ESTROUS CYCLE

Interval (days of cycle)	Number of Concentrations Mean†	± 1 SD
-1 to 2	5.17[a]	1.94
3 to 6	2.33[c,d]	1.86
7 to 10	4.83[a,b]	1.47
11 to 14	3.16[a,b,c]	2.23
15 to -2	1.00[d]	.89

† Means with same superscript letter were not significantly different (P>.05).

ANALYSIS OF VARIANCE TABLE

Source	df	SS	MS	F
Block	5	32.36	6.46	2.97*
Interval	4	72.47	18.12	8.33**
Error	20	43.53	2.18	

* P<.05.

** P<.01.

Table 18

THE NUMBER, MAGNITUDE AND DURATION OF FOLLICLE-STIMULATING HORMONE PEAKS DURING THE ESTROUS CYCLE OF THE CONTROL AND SHAM OPERATED SWINE

Variable[Δ]	Control		Sham		Control + Sham			
	Mean ± 1 SD		Mean ± 1 SD		Mean ± 1 SD		t[†]	df
No. of Peaks								
PoOvFSH	1.00	--	1.00	--	1.00	--	--	--
$\bar{x}$MCFSH	1.40	.55	.60	.89	1.00	.82	1.71	8
Magnitude (ng/ml)								
PoOvFSH	9.82	2.68	15.80	12.33	13.14	9.42	.94	7
PoOvFSH - BL	4.10	1.37	11.67	12.25	8.30	9.57	1.21	7
$\bar{x}$MCFSH	10.02	4.23	9.12	3.57	9.76	3.78	.26	5
$\bar{x}$MCFSH - BL	4.30	3.10	4.22	2.02	4.28	2.66	.03	5
Duration[▽] (hrs)								
PoOvFSH	62.37	7.72	54.81	21.26	58.17	16.25	.67	7
$\bar{x}$MCFSH	17.01	6.01	20.79	2.67	18.09	5.36	.92	5

† The t-test was used to test for significant differences between the control and sham treated swine. A pooled mean and standard deviation were not computed if t was significant at the 5% level.

Δ Abbreviations: PoOvFSH, postovulatory FSH peak; $\bar{x}$MCFSH, average mid-cycle FSH peak; BL, baseline.

▽ Duration estimates were computed in relation to baseline.

Table 19

A SUMMARY OF THE GENERAL BIOLOGICAL DATA RELATING TO PLASMA FSH HORMONE CONCENTRATIONS IN CONTROL AND SHAM OPERATED SWINE

	Control	Sham	Control + Sham		
Variable[Δ]	Mean ± 1 SD	Mean ± 1 SD	Mean± 1 SD	t[†]	df
Cycle length (days)	19.83 1.33	20.50 .55	20.17 1.03	1.14	10
Baseline (ng/ml)	5.38 1.55	4.13 1.46	4.76 1.56	1.31	8
Baseline SD (ng/ml)	1.67 .66	1.65 .60	1.66 .60	.06	8
Grand Mean[∇] (ng/ml)	5.49 1.60	4.43 1.52	4.96 1.57	1.08	8
Grand Mean SD (ng/ml)	1.83 .63	2.64 1.88	2.23 1.39	.92	8
Median[∇] (ng/ml)	5.34 1.50	4.22 1.53	4.78 1.55	1.15	8
Mode[∇] (ng/ml)	5.46 2.97	3.98 2.08	4.72 2.54	.91	8
Conc. BL + 2 SD (conc./ estrous cycle)	3.60 2.51	4.40 1.82	4.00 2.11	.58	8

† The t-test was used to test for significant differences between the control and sham treated swine. A pooled mean and standard deviation were not computed if t was significant at the 5% level.

Δ Abbreviations: BL, baseline; SD, standard deviation.

∇ Computed from all hormone concentrations (e. g., LH, FSH or PRL) collected during one estrous cycle.

Table 20

TIME INTERVAL (DAYS) BETWEEN LH AND FSH PEAKS WITHIN THE ESTROUS CYCLE OF THE CONTROL AND SHAM OPERATED SWINE

Interval Between Peaks[▽]		Control		Sham		Control + Sham			
From	To	Mean ± 1 SD		Mean ± 1 SD		Mean ± 1 SD		t[Δ]	df
PrOvLH	PoOvFSH	2.46	.49	2.90	.26	2.66	.45	1.76	9
PrOvLH	$\bar{x}$MCFSH	10.36	2.48	11.04	4.54	10.59	2.82	.25	4
PoOvFSH	$\bar{x}$MCFSH	7.86	2.90	7.89	4.21	7.87	2.93	.01	4
PoOvFSH	$\bar{x}$MCLH	6.67	.97	6.55	2.02	6.61	1.45	.12	9
$\bar{x}$MCFSH	PrOvLH	9.52	2.72	8.82	4.00	9.29	2.78	.26	9

▽ Abbreviations: PrOvLH, preovulatory LH peaks; PoOvFSH, postovulatory FSH peaks; $\bar{x}$MCFSH, average mid-cycle FSH peaks; $\bar{x}$MCLH, average mid-cycle LH peaks.

Δ The t-test was used to test for significant differences between the control and sham treated swine. A pooled mean and standard deviation were not computed if t was significant at the 5% level.

† Peak intervals were computed by measuring the distance between parallel lines drawn perpendicular to baseline from points located in the center of each peak at 1/2 peak height.

Table 21

THE EFFECT OF TIME OF DAY ON
FSH CONCENTRATIONS

Time of Day (hrs)	FSH Conc (ng/ml) Mean†	± 1 SD
0600	5.46[a,b]	1.51
1200	5.47[a,b]	1.63
1800	5.21[b]	1.56
2400	5.78[a]	1.69

† Means with same superscript letter were not significantly different (P>.05).

ANALYSIS OF VARIANCE TABLE

Source	df	SS	MS	F
Block	4	40.30	10.07	167.90**
Time of Day	3	.82	.27	4.55*
Error	12	.72	.06	

* P<.05.

** P<.01.

Table 22

THE DISTRIBUTION OF PLASMA FSH CONCENTRATIONS FOUND TO BE SIGNIFICANTLY GREATER THAN BASELINE IN RELATION TO THE DAY OF THE ESTROUS CYCLE

Interval (days of cycle)	Number of Concentrations		
	Mean†	±	1 SD
-1 to 2	.40[a]		.89
3 to 6	2.00[a]		2.35
7 to 10	.40[a]		.55
11 to 14	.80[a]		1.30
15 to -2	0.00[a]		0.00

† Means with same superscript letter were not significantly different (P>.05).

ANALYSIS OF VARIANCE TABLE[Δ]

Source	df	SS	MS	F
Block	4	1.96	.49	.25
Interval	4	8.76	2.19	1.12
Error	16	31.24	1.95	

Δ The data were coded by adding 1 FSH concentration to the sum of each collection interval, in order to eliminate 0 values during several collection intervals.

Table 23

A SUMMARY OF THE GENERAL BIOLOGICAL DATA RELATING TO PLASMA PRL CONCENTRATIONS IN CONTROL AND SHAM OPERATED SWINE

	Control		Sham		Control + Sham			
Variable[Δ]	Mean ± 1 SD		Mean ± 1 SD		Mean ± 1 SD		t[†]	df
Cycle length (days)	19.83	1.33	20.50	.55	20.17	1.03	1.14	10
Baseline (ng/ml)	6.16	.94	6.93	1.56	6.54	1.29	1.08	10
Baseline SD (ng/ml)	.86	.09	1.34	.64	1.10	.51	1.83	10
Grand Mean [∇] (ng/ml)	6.95	1.06	7.62	1.49	7.28	1.28	.89	10
Grand Mean SD (ng/ml)	2.46	1.29	2.63	1.28	2.55	1.23	1.65	10
Median [∇] (ng/ml)	6.20	.96	7.04	1.47	6.62	1.26	1.18	10
Mode [∇] (ng/ml)	5.87	.90	6.37	1.67	6.12	1.30	.65	10
Conc.>BL + 2 SD (conc./ estrous cycle	11.50	5.56	11.33	7.06	11.42	6.04	.05	10

† The t-test was used to test for significant differences between the control and sham treated swine. A pooled mean and standard deviation were not computed if t was significant at the 5% level.

Δ Abbreviations: BL, baseline; SD, standard deviation.

∇ Computed from all hormone concentrations (e. g., LH, FSH or PRL) collected during one estrous cycle.

Table 24

THE NUMBER, MAGNITUDE AND DURATION OF PRL PEAKS DURING THE ESTROUS CYCLE OF CONTROL AND SHAM OPERATED SWINE

Variable[Δ]	Control		Sham		Control + Sham			
	Mean	± 1 SD	Mean	± 1 SD	Mean	± 1 SD	t[†]	df
No. of Peaks								
PrOvPRL	2.50	1.05	2.67	1.21	2.58	1.08	.25	10
FoPRL	3.17	1.60	3.00	1.26	3.08	1.38	.20	10
Magnitude (ng/ml)								
PrOvPRL	10.30	1.88	12.69	3.53	11.49	2.97	1.46	10
PrOvPRL - BL	4.14	1.99	5.75	2.34	4.95	2.23	1.29	10
PrOvPRL - (BL+2 SD)	2.42	1.97	3.07	1.98	2.74	1.92	.57	10
FoPRL	13.70	4.02	14.86	4.82	14.28	4.27	.45	10
FoPRL - BL	7.54	3.65	7.93	4.31	7.73	3.81	.17	10
FoPRL - (BL+2 SD)	5.82	3.51	5.24	4.15	5.53	3.68	.26	10
Duration[▽] (hrs)								
PrOvPRL	20.21	4.18	20.87	4.21	20.54	4.02	.27	10
FoPRL	19.49	2.92	18.47	3.85	18.98	3.30	.51	10

† The t-test was used to test for significant differences between the control and sham treated swine. A pooled mean and standard deviation were not computed if t was significant at the 5% level.

Δ Abbreviations: PrOvPRL, preovulatory PRL peak; FoPRL, follicular PRL peak; BL, baseline; BL + 2 SD, baseline + 2 standard deviations.

▽ Duration estimates were computed in relation to baseline or baseline + 2 standard deviations.

Table 25

TIME INTERVAL (DAYS) BETWEEN LH, FSH AND PRL PEAKS WITHIN THE ESTROUS CYCLE OF THE CONTROL AND SHAM OPERATED SWINE

Interval† Between Peaks▽		Control		Sham		Control + Sham			
From	To	Mean ± 1 SD		Mean ± 1 SD		Mean ± 1 SD		t△	df
PrOvLH	PrOvPRL	.41	.40	.35	.45	.38	.41	.25	10
PrOvLH	FoPRL	15.59	1.01	17.01	.74	--	--	2.77*	10
PrOvPRL	PoOvFSH	2.18	.48	2.43	.61	2.31	.54	.77	10
PoOvFSH	FoPRL	13.23	1.47	14.23	.95	13.73	1.28	1.40	10
PrOvPRL	FoPRL	15.44	.87	17.00	.77	--	--	3.28**	10
FoPRL	PrOvLH	4.05	1.10	3.64	.76	3.86	.92	.78	10
FoPRL	PrOvPRL	4.91	.75	4.17	.95	4.54	.90	1.49	10
FoPRL	PoOvFSH	6.84	.45	6.44	.84	6.64	.67	1.03	10
Mean of consecutive adjacent mid-cycle peaks		.86	.26	.94	.35	.90	.31	.37	10

▽ Abbreviations: PrOvLH, preovulatory LH peak; PrOvPRL, preovulatory PRL peak; FoPRL, follicular PRL peak; PoOvFSH, postovulatory FSH peak.

△ The t-test was used to test for significant differences between the control and sham treated swine. A pooled mean and standard deviation were not computed if t was significant at the 5% level.

† Peak intervals were computed by measuring the distance between parallel lines drawn perpendicular to baseline from points located in the center of each peak at 1/2 peak height.

* $P<.05$.

** $P<.01$.

Table 26

THE EFFECT OF TIME OF DAY ON PRL PLASMA CONCENTRATIONS

Time of Day (hrs)	PRL Conc (ng/ml) Mean† ± 1 SD
0600	6.93[a] .93
1200	6.80[a] 1.01
1800	7.17[a] 1.29
2400	6.92[a] 1.12

† Means with same superscript letter were not significantly different ($P>.05$).

ANALYSIS OF VARIANCE TABLE

Source	df	SS	MS	F
Block	5	22.44	4.49	41.58**
Time of Day	3	.42	.14	1.30
Error	15	1.62	.11	

** $P<.01$.

Table 27

THE DISTRIBUTION OF PLASMA PRL CONCENTRATIONS FOUND TO BE SIGNIFICANTLY GREATER THAN BASELINE IN RELATION TO THE DAY OF THE ESTROUS CYCLE

Interval (days of cycle)	Number of Concentrations Mean†	± 1 SD
-1 to 2	4.00[a]	2.53
3 to 6	.00[b]	0.00
7 to 10	.17[b]	.41
11 to 14	.83[b]	1.60
15 to -2	6.17[a]	2.79

† Means with same superscript letter were not significantly different (P>.05).

ANALYSIS OF VARIANCE TABLE[Δ]

Source	df	SS	MS	F
Block	5	26.57	5.31	1.83
Interval	4	178.87	44.72	15.44**
Error	20	57.93	2.90	

Δ The data were coded by adding 1 prolactin concentration to the sum of each collection interval, in order to eliminate 0 values during several collection intervals.

** P<.01.

Table 28

LH, FSH AND PRL BASELINE CONCENTRATIONS IN THE CONTROL, SHAM, OVARIECTOMIZED AND HYSTERECTOMIZED SWINE

	LH (ng/ml)		FSH (ng/ml)		PRL (ng/ml)	
Treatment	Mean†	± 1 SD	Mean†	± 1 SD	Mean†	± 1 SD
Control	1.18[b]	.31	5.38[b]	1.56	6.16[a]	.94
Sham	1.21[b]	.19	4.52[b]	1.63	6.93[a]	1.56
Ovariectomized	2.22[a]	.14	12.20[a]	2.90	6.70[a]	2.10
Hysterectomized	1.12[b]	.34	5.30[b]	2.09	6.05[a]	1.89

† Means with same superscript letter are not significantly different (P>.05).

Table 29

LH, FSH AND PRL BASELINE CONCENTRATIONS IN THE CONTROL, SHAM, OVARIECTOMIZED AND HYSTERECTOMIZED SWINE

	ANALYSIS OF VARIANCE TABLE				
Hormone	Source	df	SS	MS	F
LH	Block	5	.27	.05	.74
	Surgery	3	4.96	1.65	23.11**
	Error	15	1.07	.07	
FSH	Block	4	42.26	10.57	4.35*
	Surgery	3	193.31	64.44	26.52**
	Error	12	29.13	2.43	
PRL	Block	5	25.19	5.04	2.41
	Surgery	3	3.25	1.08	.52
	Error	15	31.38	2.09	

* $P<.05$.

** $P<.01$.

Table 30

ESTIMATES OF BASAL PLASMA LH, FSH AND PRL CONCENTRATIONS IN OVARIECTOMIZED AND HYSTERECTOMIZED SWINE

Variable[Δ]	LH (ng/ml) Mean ± 1 SD		FSH (ng/ml) Mean ± 1 SD		PRL (ng/ml) Mean ± 1 SD	
Ovariectomized:						
Baseline	2.21	.14	12.20	2.90	6.70	2.10
Baseline SD	.47	.06	2.40	.84	.92	.31
Grand Mean[∇]	2.26	.15	12.26	2.92	6.88	1.95
Grand Mean SD	.52	.04	2.45	.86	.98	.21
Median[∇]	2.22	.18	12.57	3.05	6.70	2.13
Mode[∇]	1.96	.10	10.98	2.76	6.80	2.31
Hysterectomized:						
Baseline	1.12	.34	5.30	2.09	6.05	1.89
Baseline SD	.24	.05	1.19	2.80	1.11	.64
Grand Mean[∇]	1.37	.25	5.36	2.05	6.18	1.82
Grand Mean SD	.57	.13	1.24	.31	1.40	.58
Median[∇]	1.19	.29	5.37	2.14	6.00	1.84
Mode[∇]	.97	.37	5.30	2.39	6.33	2.86

Δ Abbreviation: SD, standard deviation.

∇ Computed from all hormone concentrations (e. g., LH, FSH or PRL) collected during the 20-day collection period.

Table 31

LH, FSH AND PRL STANDARD DEVIATION ESTIMATES OF THE BASAL HORMONE POPULATION IN THE CONTROL, SHAM OVARIECTOMIZED AND HYSTERECTOMIZED SWINE

	ANALYSIS OF VARIANCE TABLE				
Hormone	Source	df	SS	MS	F
LH	Block	5	.08	.02	8.66**
	Surgery	3	.22	.07	40.61**
	Error	15	.03	.00	
FSH	Block	4	11.83	2.96	6.43**
	Surgery	3	13.75	4.58	9.96**
	Error	12	5.46	.46	
PRL	Block	5	.59	.12	.44
	Surgery	3	.86	.29	1.08
	Error	15	4.03	.27	

** $P<.01$.

Table 32

LH, FSH AND PRL STANDARD DEVIATION ESTIMATES OF THE BASAL HORMONE POPULATION IN THE CONTROL, SHAM, OVARIECTOMIZED AND HYSTERECTOMIZED SWINE

	LH (ng/ml)		FSH (ng/ml)		PRL (ng/ml)	
Treatment	Mean†	± 1 SD	Mean†	± 1 SD	Mean†	± 1 SD
Control	.30[b]	.11	1.67[a]	.66	.86[a]	.09
Sham	.24[b]	.07	1.65[a]	.60	1.34[a]	.64
Ovariectomized	.47[a]	.06	2.40[a]	.85	.92[a]	.31
Hysterectomized	.25[b]	.05	1.19[a]	.28	1.11[a]	.64

† Means with same superscript letter are not significantly different ($P>.05$).

Table 33

NUMBER OF LH, FSH AND PRL CONCENTRATIONS WHICH WERE SIGNIFICANTLY GREATER THAN BASELINE IN THE CONTROL, SHAM, OVARIECTOMIZED AND HYSTERECTOMIZED SWINE

	LH		FSH		PRL	
Treatment	Mean†	± 1 SD	Mean†	± 1 SD	Mean†	± 1 SD
Control	16.50[a]	5.68	3.60[a]	2.51	11.50[a]	5.50
Sham	17.17[a]	4.12	2.60[a]	1.95	11.33[a]	7.06
Ovariectomized	4.17[b]	2.23	.20[b]	.45	3.17[b]	2.40
Hysterectomized	19.00[a]	6.63	4.40[a]	1.82	4.17[b]	2.40

† Means with same superscript letter are not significantly different (P>.05).

Table 34

NUMBER OF LH, FSH AND PRL CONCENTRATIONS WHICH WERE SIGNIFICANTLY GREATER THAN BASELINE IN THE CONTROL, SHAM, OVARIECTOMIZED AND HYSTERECTOMIZED SWINE

	ANALYSIS OF VARIANCE TABLE				
Hormone	Source	df	SS	MS	F
LH	Block	5	191.75	38.35	1.92
	Surgery	3	826.83	275.61	13.81**
	Error	15	299.42	19.96	
FSH	Block	4	42.95	10.74	11.31**
	Surgery	3	49.80	16.60	17.47**
	Error	12	11.45	.95	
PRL	Block	5	275.75	55.15	4.53*
	Surgery	3	363.50	121.17	9.95**
	Error	15	182.75	12.18	

* $P<.05$.

** $P<.01$.

Table 35

THE NUMBER, MAGNITUDE AND DURATION OF LH, FSH AND PRL PEAKS IN THE OVARIECTOMIZED ANIMALS

Hormone	Variable†	Mean	± 1 SD
LH	No. of Peaks		
	Ovar LH	4.00	2.10
	Magnitude (ng/ml)		
	Ovar LH	3.47	.21
	Ovar LH - BL	1.26	.09
	Ovar LH - (BL + 2 SD)	.31	.05
	Duration$^{\Delta}$ (hrs)		
	Ovar LH	14.20	2.46
FSH	No. of Peaks		
	Ovar FSH	.40	.55
	Magnitude (ng/ml)		
	Ovar FSH	21.43	.82
	Ovar FSH - BL	6.59	1.15
	Ovar FSH - (BL + 2 SD)	.78	--
	Duration$^{\Delta}$ (hrs)		
	Ovar FSH	20.33	18.06
PRL	No. of Peaks		
	Ovar PRL	2.67	1.97
	Magnitude (ng/ml)		
	Ovar PRL	9.32	2.00
	Ovar PRL - BL	2.61	.95
	Ovar PRL - (BL + 2 SD)	.86	1.09
	Duration$^{\Delta}$ (hrs)		
	Ovar PRL	16.93	5.86

† Abbreviations: Ovar LH, ovariectomy LH peak; Ovar FSH, ovariectomy FSH peak; Ovar PRL, ovariectomy PRL peak.

Δ Duration estimates made in relation to baseline.

Table 36

THE EFFECT OF TIME OF DAY ON PLASMA LH, FSH AND PRL CONCENTRATIONS IN 6 OVARIECTOMIZED SWINE

Hormone	Time (hrs)	Mean† (ng/ml)	± 1 SD
LH	0600	$14.02^{a,b}$	2.34
	1200	$13.25^{b,c}$	2.01
	1800	11.99^{c}	2.00
	2400	14.86^{a}	2.47
FSH	0600	12.38^{a}	3.08
	1200	12.42^{a}	3.29
	1800	11.95^{a}	2.79
	2400	12.30^{a}	2.58
PRL	0600	6.78^{a}	1.89
	1200	6.90^{a}	2.24
	1800	6.65^{a}	2.10
	2400	6.76^{a}	2.11

† Means with same superscript letter are not significantly different ($P>.05$).

Table 37

THE EFFECT OF TIME OF DAY ON PLASMA
LH, FSH AND PRL CONCENTRATIONS
IN 6 OVARIECTOMIZED SWINE

	ANALYSIS OF VARIANCE TABLE				
Hormone	Source	df	SS	MS	F
LH	Animal	5	.46	.09	7.13**
	Time	3	.74	.25	19.37**
	Error	15	.19	.01	
FSH	Animal	4	136.06	34.00	145.00**
	Time	3	.70	.23	.99
	Error	12	2.82	.23	
PRL	Animal	5	86.17	17.23	231.00**
	Time	3	.15	.05	.68
	Error	15	1.12	.07	

** P<.01.

Table 38

THE EFFECT OF SURGICAL TREATMENT AND COLLECTION INTERVAL ON THE NUMBER OF LH CONCENTRATIONS WHICH WERE SIGNIFICANTLY GREATER THAN BASELINE

Treatment	Interval† (Days)	Mean Δ (Conc.>BL)	1 SD
Control	-1 to 2	5.17[a]	1.94
	3 to 6	2.33[b,c]	1.86
	7 to 10	4.83[a,b]	1.47
	11 to 14	3.17[a,b,c]	2.23
	15 to -2	1.00[c]	.89
Sham	-1 to 2	6.67[a]	1.97
	3 to 6	2.33[b]	2.16
	7 to 10	2.00[b]	1.26
	11 to 14	3.00[b]	1.55
	15 to -2	3.17[b]	1.60
Ovariectomized	-1 to 2	1.00[a]	1.55
	3 to 6	1.17[a]	1.47
	7 to 10	1.00[a]	1.55
	11 to 14	.17[a]	.41
	15 to -2	.83[a]	1.17
Hysterectomized	-1 to 2	6.00[a]	2.10
	3 to 6	3.33[b]	3.08
	7 to 10	2.67[b]	1.86
	11 to 14	2.33[b]	1.21
	15 to -2	4.67[a,b]	1.63

Δ LH concentrations within a 4-day collection interval which were significantly greater than baseline. Means with same superscript letter were not significantly different (P>.05).

† Time estimates for the ovariectomized and hysterectomized treatments were based on predicted cycle length of 20 days. Time estimates were computed in relation to the day of the estrous cycle on which surgery was performed.

Table 39

THE EFFECT OF SURGICAL TREATMENT AND COLLECTION INTERVAL ON THE NUMBER OF FSH CONCENTRATIONS WHICH WERE SIGNIFICANTLY GREATER THAN BASELINE

Treatment	Interval† (Days)	Mean$^{\Delta}$ (Conc.>BL)	1 SD
Control	-1 to 2	.40^{a}	.89
	3 to 6	2.00^{a}	2.35
	7 to 10	.40^{a}	.55
	11 to 14	.80^{a}	1.30
	15 to -2	0.00^{a}	0.00
Sham	-1 to 2	.20^{b}	.45
	3 to 6	3.00^{a}	1.87
	7 to 10	.40^{b}	.89
	11 to 14	0.00^{b}	0.00
	15 to -2	.80^{a,b}	1.10
Ovariectomized	-1 to 2	0.00^{a}	0.00
	3 to 6	0.00^{a}	0.00
	7 to 10	.20^{a}	.45
	11 to 14	0.00^{a}	0.00
	15 to -2	0.00^{a}	0.00
Hysterectomized	-1 to 2	.20^{a}	.45
	3 to 6	0.00^{a}	0.00
	7 to 10	.40^{a}	.89
	11 to 14	1.40^{a}	1.67
	15 to -2	.60^{a}	.89

Δ FSH concentrations within a 4-day collection interval which were significantly greater than baseline. Means with same superscript letter were not significantly different (P>.05).

† Time estimates for the ovariectomized and hysterectomized treatments were based on predicted cycle length of 20 days. Time estimates were computed in relation to the day of the estrous cycle on which surgery was performed.

Table 40

THE EFFECT OF SURGICAL TREATMENT AND COLLECTION INTERVAL ON THE NUMBER OF PRL CONCENTRATIONS WHICH WERE SIGNIFICANTLY GREATER THAN BASELINE

Treatment	Interval† (Days)	Mean Δ (Conc.>BL)	1 SD
Control	-1 to 2	4.17[a,b]	2.86
	3 to 6	.17[c]	.41
	7 to 10	.17[c]	.41
	11 to 14	.83[c]	1.60
	15 to -2	6.17[a]	2.79
Sham	-1 to 2	5.50[a]	4.46
	3 to 6	.33[c]	.52
	7 to 10	0.00[c]	0.00
	11 to 14	.17[c]	.41
	15 to -2	5.33[a,b]	2.66
Ovariectomized	-1 to 2	.50[a]	.55
	3 to 6	.17[a]	.41
	7 to 10	1.00[a]	.63
	11 to 14	.67[a]	.82
	15 to -2	.83[a]	1.33
Hysterectomized	-1 to 2	.33[a]	.52
	3 to 6	1.17[a]	.98
	7 to 10	2.00[a]	1.90
	11 to 14	.33[a]	.52
	15 to -2	.33[a]	.52

Δ PRL concentrations within a 4-day collection interval which were significantly greater than baseline. Means with same superscript letter were not significantly different (P>.05).

† Time estimates for the ovariectomized and hysterectomized treatments were based on predicted cycle length of 20 days. Time estimates were computed in relation to the day of the estrous cycle on which surgery was performed.

Table 41

THE EFFECT OF SURGICAL TREATMENT AND COLLECTION INTERVAL ON THE NUMBER OF HORMONE CONCENTRATIONS WHICH WERE SIGNIFICANTLY GREATER THAN THE BASELINE

	ANALYSIS OF VARIANCE TABLE					
Hormone	Source	df	SS	MS	F	F'
LH	Block	5	38.35	7.67	2.77*	.85
	Interval	4	107.29	26.82	9.70**	2.98
	Surgery	3	165.37	55.12	19.93**	6.12**
	Interval x Surgery	12	108.17	9.01	3.26**	
	Error	95	262.82	2.77		
FSH	Block	4	2.94	.74	.29	
	Interval	4	13.84	3.46	1.35	
	Surgery	3	9.96	3.32	1.29	
	Interval x Surgery	12	33.84	2.82	1.10	
	Error	76	195.26	2.57		
PRL	Block	5	26.35	5.27	2.03	.27
	Interval	4	159.12	39.78	15.29**	2.04
	Surgery	3	72.70	24.23	9.32**	1.24
	Interval x Surgery	12	233.67	19.47	7.49**	
	Error	95	247.15	2.60		

* $P<.05$.

** $P<.01$.

Table 42

THE NUMBER, MAGNITUDE AND DURATION OF LH, FSH AND PRL IN THE HYSTERECTOMIZED ANIMALS

Hormone	Variable†	Mean	± 1 SD
LH	No. of Peaks		
	Hyst LH	14.30	4.59
	Magnitude (ng/ml)		
	Hyst LH C1	2.15	.30
	Hyst LH C1 - BL	1.03	.26
	Hyst LH C1 - (BL + 2 SD)	.54	.25
	Hyst LH C2	2.63	.23
	Hyst LH C2 - BL	1.51	.22
	Hyst LH C2 - (BL + 2 SD)	1.02	.23
	Duration$^{\Delta}$ (hrs)		
	Hyst LH C1	12.92	.33
	Hyst LH C2	19.50	1.49
FSH	No. of Peaks		
	Hyst FSH	2.00	1.22
	Magnitude (ng/ml)		
	Hyst FSH	8.12	1.95
	Hyst FSH - BL	2.82	.62
	Hyst FSH - (BL + 2 SD)	.45	.30
	Duration$^{\Delta}$ (hrs)		
	Hyst FSH	21.00	4.51
PRL	No. of Peaks		
	Hyst PRL	3.00	1.41
	Magnitude (ng/ml)		
	Hyst PRL	10.18	2.95
	Hyst PRL - BL	3.90	1.19
	Hyst PRL - (BL + 2 SD)	1.73	.90
	Duration$^{\Delta}$ (hrs)		
	Hyst PRL	17.69	3.50

† Abbreviations: Hyst LH, hysterectomy LH peak; Hyst FSH, hysterectomy FSH peak; Hyst PRL, hysterectomy PRL peak; BL, baseline; BL + 2 SD, baseline + 2 standard deviations.

Δ Duration estimates made in relation to baseline.

Table 43

THE EFFECT OF TIME OF DAY ON PLASMA LH, FSH AND PRL CONCENTRATIONS IN 6 HYSTERECTOMIZED SWINE

Hormone	Time (hrs)	Mean† (ng/ml)	±	1 SD
LH	0600	1.40[a,b]		.23
	1200	1.21[c]		.24
	1800	1.27[c]		.33
	2400	1.60[a]		.27
FSH	0600	5.38[a]		2.27
	1200	5.47[a]		1.97
	1800	5.17[a]		1.86
	2400	5.41[a]		2.09
PRL	0600	6.33[a,b]		2.07
	1200	6.04[a,b]		1.84
	1800	5.83[b]		1.58
	2400	6.54[a]		1.90

† Means with same superscript letter are not significantly different (P>.05).

Table 44

THE EFFECT OF TIME OF DAY ON PLASMA LH, FSH AND PRL CONCENTRATIONS IN 6 HYSTERECTOMIZED SWINE

	ANALYSIS OF VARIANCE TABLE				
Hormone	Source	df	SS	MS	F
LH	Animal	5	1.25	.25	17.05**
	Time	3	.52	.17	11.75**
	Error	15	.22	.01	
FSH	Animal	4	67.11	16.78	374.33**
	Time	3	.25	.08	1.88
	Error	12	.54	.04	
PRL	Animal	5	66.41	13.28	76.32**
	Time	3	1.74	.58	3.33*
	Error	15	2.61	.17	

* P<.05.

** P<.01.

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