To the Graduate Council:

I am submitting herewith a thesis written by Thomas Wayne Dowling entitled "The Release Patterns of Luteinizing Hormone in the Prepubertal Versus the Mature Cycling Gilt Under Continuous Infusion of Gonadotrophic Releasing Hormone as a Possible Indicator of Pituitary Maturity." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Frank B Masincupp

Frank B. Masincupp, Major Professor

We have read this thesis
and recommend its acceptance:

R L Murphree

Hugo Liles

Accepted for the Council:

L Evans Roth

Vice Chancellor
Graduate Studies and Research

Ag-VetMed
Thesis
79
.D784
cop. 2

THE RELEASE PATTERNS OF LUTEINIZING HORMONE IN THE PREPUBERTAL VERSUS THE MATURE CYCLING GILT UNDER CONTINUOUS INFUSION OF GONADOTROPHIC RELEASING HORMONE AS A POSSIBLE INDICATOR OF PITUITARY MATURITY

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Thomas Wayne Dowling

December 1979

1404867

ACKNOWLEDGEMENTS

The author gratefully acknowledges the donation of the gonadotrophic releasing hormone (GnRH) analogue given by Dr. Ranier Muser of American Hoechst Pharmaceuticals, Somerville, New Jersey.

The author wishes to thank his major professor, Dr. Frank Masincupp, for his guidance in this project, Drs. Hugo Eiler and Robert Murphree, whose doors were always open, and Dr. Hank Kattesh for his helpful suggestions. The author is very grateful to fellow students Carol Collyer, Charles Dugan, and Guy Dyer for their many hours contributed to this study.

A special thanks is extended to wife Sharon, who participated in all phases of the experiment from the beginning and provided limitless encouragement until the bitter end.

ABSTRACT

An attempt was made in this study to establish an indicator of the maturity of the pituitary and/or hypothalamo-pituitary axis by comparing the luteinizing hormone (LH) release profile under continuous infusion of gonadotrophic releasing hormone (GnRH) in the prepubertal and mature cycling gilt. This was done in an effort to show whether or not an immature pituitary gland could be responsible for failure to maintain pregnancy in the prepubertal gilt when puberty is induced.

The experiment consisted of three prepubertal gilts in one group and three cyclic gilts in a second group. GnRH was administered at a dose rate of 2.3 μg/hr given in pulses over six hours, with blood sampling taking place before, during, and after infusion. Plasma LH values were determined with a human LH radioimmunoassay kit which was shown to be able to detect porcine LH with some reliability.

Plasma LH values detected by this kit were below those levels found by some workers for the preovulatory LH surge but were within the range of values which have been reported for times other than estrus. The results showed a significant ($P < .05$) difference in the mean baseline LH values between groups and no significant ($P > .05$) difference between groups for mean LH response levels during GnRH infusion. There appears to be a greater change from mean baseline to peak LH levels in the prepubertal gilts, but the increase was not significant ($P > .05$). It was concluded that a single-peaked or monophasic LH release profile vs. a double-peaked or biphasic LH release profile was not evident in the immature and mature gilts respectively as noted in other species. This

appears to have been due to an insufficient dose rate of the releasing hormone (GnRH).

TABLE OF CONTENTS

CHAPTER		PAGE
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	3
	Puberty: A Definition	3
	Non-Reproductive Factors Affecting Puberty	3
	Stimulating Ovulation in the Prepubertal Gilt	6
	Attempts to Maintain Pregnancy with Exogenous Hormones	7
	The Role of the Uterus and Corpus Luteum Regression (Luteolysis)	8
	Influence of Embryos on Corpus Luteum Maintenance	10
	Discovering the Luteolytic Factor	11
	Uterine Specific Proteins	14
	GnRH and the Release of LH	16
	LH Levels During the Estrous Cycle	16
	Pituitary LH Stores	18
	The LH Release Profile as an Indicator of Pituitary Maturity	20
	Summary	21
III.	MATERIALS AND METHODS	22
	Animals and Management	23
	Cannulation	24
	Dosing and Blood Sampling	25
	Radioimmunoassay of LH	26
	Radioimmunoassay Validation	26
	Statistical Analysis	27
IV.	RESULTS AND DISCUSSION	29
	Radioimmunoassay Validation	29
	LH Release Patterns	34
V.	SUMMARY	46
LIST OF REFERENCES		48
VITA		59

LIST OF TABLES

TABLE		PAGE
I.	Recovery	30
II.	Parallelism	31
III.	Interassay Variations	33

LIST OF FIGURES

FIGURE		PAGE
1.	LH Profile for Pig Number 1 (Prepubertal)	35
2.	LH Profile for Pig Number 2 (Prepubertal)	36
3.	LH Profile for Pig Number 3 (Prepubertal)	37
4.	LH Profile for Pig Number 4 (Mature Cycling)	38
5.	LH Profile for Pig Number 5 (Mature Cycling)	39
6.	LH Profile for Pig Number 6 (Mature Cycling)	40
7.	Mean LH Profile for Prepubertal and Mature Cycling Gilts .	41

CHAPTER I

INTRODUCTION

Profit is the main goal and objective of practically every livestock operation. This includes the production of swine. In order to reap maximum profit from the swine breeding herd, it is critical that the female pig develops the ability to produce healthy, viable offspring at as early an age as possible.

Man has often intervened in various ways to persuade nature to do his bidding. In regard to swine reproduction, it has been shown that gilts can be induced to ovulate earlier than they normally do, but when this is done, there is little success in maintaining gestation in these pigs beyond the early stages. Several possible factors have been considered as reasons for this. These include an imbalance between the luteotropic and luteolytic mechanisms (Puglisi et al., 1978), the immaturity of the uterus (Murray and Grifo, 1976), the immaturity of the ovary (Dial et al., 1978), or the lack of luteotrophic support to maintain the corpus luteum due to the immaturity of the pituitary gland and/or the hypothalamo-pituitary axis (Segal and Baker, 1973; Segerson, 1976; Rampacek et al., 1976).

The purpose of this experiment was to explore one possible explanation as to why immature gilts fail to maintain pregnancy. The response of the pituitary gland to release luteinizing hormone (LH) over time under continuous infusion with gonadotrophic releasing hormone (GnRH) in the prepubertal gilt and the mature cycling gilt was studied. The

objective of this study was to establish whether or not the profile of LH release over time in these animals could serve as an indicator of the maturity of the pituitary gland.

CHAPTER II

REVIEW OF LITERATURE

I. PUBERTY: A DEFINITION

Puberty has been defined in a number of ways. Lasely (1968) defined it as "the period in the female's life when she becomes capable of reproduction and when the reproductive processes begin to function." Another definition from Ramirez (1973) can be quoted as "the biological phase that links maturity and immaturity." Various aspects of swine management have been studied to determine their contribution to the establishment of the female's reproductive cycle.

II. NON-REPRODUCTIVE FACTORS AFFECTING PUBERTY

Several factors outside of the reproductive system have been shown to have an influence on the attainment of puberty. These range from environmental to social influences.

Effects of Season, Boar, and Penning

Mavrogensis and Robison (1976) investigated the effects of season of birth, presence or absence of boars, and number of gilts per pen on age and weight at puberty. They reported that fall-born gilts reached puberty at an earlier age and lower weight than spring-born gilts. These findings agree with those reported earlier by Warnick et al. (1951).

In the same report, the presence of boars was found to substantially reduce age and weight at puberty. Age and weight at puberty were also substantially reduced by placing 30 gilts per pen rather than penning

gilts individually. They found significant ($P < .05$) season x boar and season x grouping interactions, however, demonstrating that for this experiment, the effect of boars and grouping held true for the spring-born gilts only.

Brooks and Cole (1970) reported that introducing a boar to 165- or 190-day old gilts brought about estrus activity. Considerable synchrony of estrus in 165-day gilts was found when boars were rotated vs. using only one boar. In either group, nonexposure to the boar resulted in fewer gilts reaching puberty. Zimmerman et al. (1969) reported that gilts exposed to a boar beginning at approximately 126 days of age reached puberty earlier than gilts first exposed at 103 days and earlier than young gilts not exposed at all.

The influence of the boar on gilts attaining puberty was thought to be similar to that in mice. Vandenbergh (1969) reported that the scent of the male mouse is, in itself, sufficient to influence the attainment of puberty in the female. Thibault et al. (1966) investigated the olfactory involvement of the boar in influencing the onset of estrus in gilts by performing bulbectomy experiments but found nothing conclusive. They did, however, report another factor affecting the onset of puberty--the stress of transporting prepubertal gilts, which they considered to be an even greater stimulus to cycling behavior than the introduction of boars. This agrees with the findings of J. G. O'Neal (personal communication).

Nutritional Considerations

Nutritional factors which have been considered to contribute to the attainment of puberty include growth rate, age, weight, and levels of energy and protein in the diet.

Robertson et al. (1951) and Cunningham et al. (1974) reported a negative correlation between average daily gain and age at puberty. That is, it was shown that faster growing gilts tended to reach puberty at an earlier age than those which grew more slowly. It has been argued, however, that faster growing gilts are heavier at puberty than other gilts reaching puberty at the same age (Lodge and McPherson, 1961; Friend, 1977). This would indicate that puberty in pigs appears to be not growth rate or body weight dependent, but rather age dependent.

The effects of restricting energy in the diet of gilts has been studied. Self et al. (1955) reported that gilts on a full-fed energy regime were older at puberty than gilts limit-fed. Sorensen et al. (1961) and Friend (1977) reported that the ages at puberty were about the same for gilts fed either low or high levels of energy. However, most workers seem to agree that full-feeding energy leads to the attainment of puberty at an earlier age than for gilts which are severely restricted in their intake of energy (Robertson et al., 1951; Self et al., 1955; Goode et al., 1960).

Manipulations of the protein content of pig rations have yielded conflicting results. Robertson et al. (1951) reported that gilts fed a 20% protein diet were older at puberty than gilts fed 11.25% protein. Cunningham et al. (1974) showed that gilts fed a 14% protein ration

reached puberty 18.7 days earlier than those fed a 10% protein diet. Friend (1977) reported no significant effect of protein levels on age at puberty.

III. STIMULATING OVULATION IN THE PREPUBERTAL GILT

Casida (1934, 1935) was among the first to show that ovulation could be induced in the immature gilt using gonadotrophic hormones. Tanabe et al. (1949) used sheep pituitary extracts as the gonadotrophic source to induce ovulation in gilts. Most of the work done in stimulating ovulation in prepubertal gilts has utilized injection of pregnant mare serum gonadotrophin (PMSG) in various dosages followed by human chorionic gonadotrophin (HCG) given 48 to 96 hours later (Dziuk and Gelbach, 1966; Baker and Coggins, 1968; Rampacek et al., 1976; Guthrie, 1978). Dzuik and Gelbach (1966) reported that 92% of 95- to 130-day old gilts can be stimulated to ovulate with PMSG and HCG. Dzuik and Baker (1962) reported that 94% of stimulated gilts will ovulate approximately 40 hours after HCG injection.

These reports document the fact that ovulation can be induced in the prepubertal gilt. However, a major problem has been the failure of the stimulated gilt to maintain pregnancy beyond 25 days of gestation. Squiers et al. (1952) and Lerner et al. (1957) reported that the most critical period for embryonic survival in swine occurs, in general, during the first 25 days of gestation.

IV. ATTEMPTS TO MAINTAIN PREGNANCY WITH EXOGENOUS HORMONES

It is well established that progesterone is the major steroid hormone responsible for the maintenance of pregnancy in mammals. The main source of progesterone is the corpus luteum.

Several experiments have been conducted to study the effects of exogenous hormones on reducing the rates of embryo mortality in swine. Sammelwitz et al. (1956) showed a decrease in embryonic survival in rats and pigs treated with exogenous progesterone. Spies et al. (1959) reported that exogenous progesterone had no beneficial effect on embryo survival in intact cycling gilts and found better survival rates in some controls. Both studies reported that the corpora lutea regressed when exogenous progesterone was administered. Haines et al. (1957) reported no improvement in embryo survival with exogenous progesterone. Neville et al. (1971) reported only limited success when progesterone was given every ten days beginning on day 19 of gestation.

Gardner et al. (1963) demonstrated that exogenous estrogens could maintain the function but not the size of the corpora lutea in gilts. Day et al. (1959) reported that neither estrogen nor progesterone when given alone could maintain pregnancy and showed a need for both hormones. That report conflicts with that of Reddy et al. (1957), who reported greater embryo mortality in gilts treated with a combination of progesterone and estrogen together. Gentry et al. (1973) reported that when progesterone is in limiting concentrations compared to estrogen, increasing estrogen had an adverse effect on embryo survival.

V. THE ROLE OF THE UTERUS AND CORPUS LUTEUM REGRESSION (LUTEOLYSIS)

The uterus is known to exert an influence on the lifespan of the corpus luteum. Chu et al. (1945) showed that the lifespan of the corpus luteum of the rabbit could be extended by removal of the uterus. Wiltbank and Casida (1956) reported that complete removal of the uterus in the ewe and cow would lengthen the lifespan of the corpus luteum up to 100 and 154 days respectively. Corpus luteum persistence was also demonstrated in the hysterectomized beef heifer (Anderson et al., 1962). The effect of hysterectomy in maintaining corpus luteum function has been demonstrated several times in prepubertal and mature gilts (Salisbury et al., 1975; Rampacek et al., 1976; Puglisi et al., 1978). Anderson et al. (1969) reported that removal or destruction of endometrial tissue alone induced maintenance of the corpus luteum.

Spies et al. (1960) demonstrated that when the uterus was removed, corpus luteum regression still occurred when exogenous progesterone was administered to gilts. They proposed that the hormone treatment blocked the release of a corpus luteum maintaining substance from the pituitary gland. It is well to note that in the pigs, the corpus luteum attains its maximal size and rate of progesterone production between days 12 to 15 of the estrus cycle, after which time it will regress (Duncan et al., 1960; Tillson and Erb, 1967; Cavazos et al., 1969; Stabenfeldt et al., 1969; Henricks et al., 1972). Sammelwitz et al. (1961) reported no effect of progesterone administration on the developing corpus luteum when given from day of ovulation to day 10 to 13, but when given on day

12 to 16 and beyond, the normally formed corpora lutea rapidly degenerated. They proposed that progesterone treatments inhibited LH release and subsequent maintenance of the corpora lutea during pregnancy.

In pregnant gilts treated with rabbit serum containing anti-ovine LH antibodies, it was demonstrated that the corpora lutea atrophied, and all of the embryos were lost (Spies et al., 1967). In sheep, it was reported that continuous infusion of LH prolonged the lifespan of the corpus luteum, thus demonstrating its effect on overcoming the luteolytic effect of the uterus (Karsch et al., 1971).

Staigmiller et al. (1974) reported that pigs unilaterally ovariectomized on day 15 of gestation responded with compensatory growth of the remaining luteal tissue, which subsequently increased the production of progesterone after a period of 12 days. This appears to substantiate earlier claims that a feedback relationship between the ovaries and pituitary develops when the corpora lutea are maintained beyond their normal cyclic lifespan in mature gilts.

Segal and Baker (1973) reported some success in improving embryo survival in stimulated prepubertal gilts by administering daily dosages of HCG from day 8 to 25 of gestation. Segerson (1976) showed that corpus luteum function can be maintained consistently when HCG is given to stimulated prepubertal gilts. The same report demonstrated that luteinizing releasing hormone (LRH or GnRH) is incapable of maintaining the corpora lutea in the stimulated prepubertal gilt.

A lack of luteotrophic support necessary to maintain the corpus luteum of pregnancy has been implicated as a possible reason for the

inability of the prepubertal pig to maintain pregnancy (Segal and Baker, 1973; Segerson, 1976; Rampacek et al., 1976). It has also been suggested that this is overcome with maturity when the pituitary may be more capable of providing the necessary luteotrophic support (Segal and Baker, 1973; Rampacek et al., 1976). The hysterectomy experiments of Rampacek et al. (1976) and Puglisi et al. (1978) contradict these hypotheses and suggest the inability of the prepubertal gilt to maintain pregnancy is not completely due to a lack of luteotrophic support, but rather to an imbalance between the lytic and trophic mechanisms. Puglisi et al. (1978) provided evidence supporting this theory. They showed that in prepubertal gilts a subtotal hysterectomy in which 15% of one uterine horn or body remained intact, luteal regression was observed. Conversely, luteal regression was not observed in mature gilts unless 25% of one uterine horn was intact.

VI. INFLUENCE OF EMBRYOS ON CORPUS LUTEUM MAINTENANCE

Embryos themselves have been shown to have an effect on maintaining pregnancy and the integrity of the corpus lutrum. Polge et al. (1966) reported that only one embryo was capable of maintaining pregnancy in pigs but only when all of the uterus except that supporting the embryo was removed. They demonstrated that it takes at least four embryos to overcome the luteolytic effect exerted by the uterus.

Dhindsa and Dziuk (1968) demonstrated that in gilts, removing the embryos from one uterine horn between days 4 to 10 of gestation resulted in cessation of pregnancy. They concluded that embryos must be present

in both horns until day 10 to 12 for gestation to continue, but embryos are required in only one horn after day 12. Niswender et al. (1970) reported that gilts made unilaterally pregnant on day 2 of gestation maintained the corpora lutea on the ovary adjacent to the gravid horn, but maintenance of the corpora lutea on both ovaries was only possible when the gilts were made unilaterally pregnant after day 12. It was concluded that the signal for corpus luteum maintenance was mediated unilaterally. Moor and Rowson (1966) reported similar findings in sheep.

Infusions of embryo homogenates into the uterus have provided further information regarding the role of embryos and corpus luteum maintenance. Rowson and Moor (1967) reported that daily infusions of homogenates of day 14 to 15 sheep embryos into the uterus of nonpregnant sheep significantly lengthened their estrous cycle. Longnecker and Day (1972) showed that unilaterally pregnant gilts had higher pregnancy rates when the non-gravid horn was infused twice daily with 20 to 25 or 35 to 40 day embryo homogenates.

Segal and Baker (1973) reported that the corpora lutea of pregnant prepubertal gilts stimulated to ovulate with PMSG and HCG and subsequently treated again with HCG to enhance corpus luteum function produced more progesterone per gram of tissue than similarly treated gilts that were hysterectomized. This indicated that embryos could have prevented the luteolytic effect of the uterus.

VII. DISCOVERING THE LUTEOLYTIC FACTOR

Uterine Flushings

Several studies have reported that a luteolytic substance is found in uterine flushings. Schomberg (1967) demonstrated <u>in vitro</u> the luteolytic

activity of pig uterine flushings taken from various stages of the estrous cycle on cultured granulosa cells. He reported that flushings obtained on days 1 to 10 and day 20 had no detrimental effect on the growth of granulosa cells or their production of progesterone. However, it was also found that flushings from day 12 were luteolytic, and day 14 to 18 flushings were even more so. It has been reported that the appearance of luteolytic activity in uterine flushings coincides with a decline in progesterone production (Gomes et al., 1965; Masuda et al., 1967).

Christensen and Day (1972) showed that pigs made unilaterally pregnant and accessory corpora lutea induced with PMSG and HCG showed unilateral regression of the corpora lutea adjacent to the non-gravid horn when uterine flushing from various days of the estrous cycle and early pregnancy were infused into that horn. They postulated that pregnancy prevents luteal regression by counteracting the luteolytic influence rather than preventing its development.

Prostaglandin F2α

Prostaglandin F2α (PGF2α) is a derivative of arachadonic acid. It has been found to be the luteolytic factor in ewes (Wilson et al., 1972); cows (Liehr et al, 1972; Lauderdale, 1972); and mares (Douglas and Ginther, 1972). These studies showed that by administering PGF2α, the estrous cycles of these species could be shortened and even synchronized to some extent in the bovine. Diehl et al. (1974) showed that intramuscular injection of PGF2α administered to pregnant swine induced labor within 28.9 $\pm$ 1.8 hours after injection. A marked drop in plasma progesterone levels accompanied this response. Diehl and Day (1974) demonstrated

that progesterone levels decreased when PGF2α was given early in gestation suggesting that PGF2α may initiate parturition by its luteolytic action on the corpora lutea of pregnant gilts. Gleeson and Thorburn (1973) gave evidence that endogenous prostaglandin F reaches peak concentration in the sow when progesterone levels are lowest during luteolysis which takes place prior to estrus.

Hallford et al. (1975) showed that treating pigs with PGF2 on day 12 and 13 of the cycle shortened the cycle length and resulted in decreased plasma progesterone levels. Kryzmowski et al. (1978) reported that PGF2α infused into the anterior uterine vein of pigs at different times of the cycle resulted in a drastic drop in progesterone levels when treated at days 12, 14, and 15. The expected progesterone peak at day 13 was not found in pigs treated on day 6, and these same pigs demonstrated inhibition of luteal growth.

Prostaglandin dehydrogenases (enzymes involved with prostaglandin catabolism) have been found to be active in the human placenta (Jarabak, 1972; Schlegel et al., 1974; Braithwaite and Jarabak, 1975), which has been suggested as a major site of prostaglandin metabolism during pregnancy (Jarabak, 1972). Niimura and Ishida (1978) reported that NAD-dependent 15-hydroxyprostaglandin dehydrogenase (PGDH) was active in the uterine and chorionic epithelia of pigs at day 30, 60, and 90 when prostaglandins of the A, E, or F series were used as substrate. These results indicated that the PGDH enzymes may be involved in the mechanism by which embryos counteract the luteolytic mechanism of the uterus.

VIII. UTERINE SPECIFIC PROTEINS

It has long been known that the uterine environment influences the development of the embryo. Krishnan and Daniel (1967) were the first to demonstrate a specific uterine protein originating from the uterus of the rabbit, which they called "blastokinin." They suggested that this protein, which is found at about the time of blastulation, is stimulatory to growth and development of the rabbit embryo. A specific uterine protein has also been found in other species, including the pig, in which it has been referred to as "purple" or "lavender pig protein" (Murray et al., 1972).

Several studies have reported changes in the uterine protein secretion during the estrous cycle and pregnancy. Murray et al. (1971) reported that three protein fractions occur throughout the estrous cycle of the pig, varying only in their respective proportions throughout the cycle. They reported an additional protein (fraction V) beginning at day 9 and a purplish glycoprotein (fraction IV) which appeared between days 14 and 16. Murray et al. (1972) later reported that the amount of total uterine protein was relatively constant during the first nine days of the cycle, began to increase by day 10, reached maximum concentrations by day 15, and then declined sharply on days 17, 18, and 20 to levels found prior to day 10. Protein fraction IV, the purple or lavender pig protein, first appeared at day 12 and was not present before day 9 or after day 16. Squire et al. (1972) reported similar findings on days 2 to 11, with additional proteins found by day 12. Progesterone has been shown to be the major hormone responsible for the changes in uterine proteins during the estrous cycle of swine (Knight et al., 1973).

The definite role of uterine proteins has not been conclusively determined. It has been reported, however, that the lavender fraction IV uterine protein is involved with placental and, hence, fetal development (Chen and Bazer, 1973; Knight et al., 1974). Chen and Bazer (1973) reported a significant reduction in placental length and allantoic fluid protein concentration at day 30 in gilts treated at day 7 with antiserum to the lavender fraction IV uterine protein. Treatment later in gestation resulted in significant reduction to fetal length and weight. Knight et al. (1974) reported an increase in day 15 uterine secretory activity in gilts treated with exogenous progesterone and enhanced placental development manifested as an increased allantoic fluid volume and increased placental surface area.

Chen et al. (1975) utilized fluorescent antibody techniques to demonstrate that the epithelium of the oviduct and uterine glands are the probable source of porcine purple protein. They reported low fluorescence between days 0 to 9 of the estrous cycle, with high degrees of fluorescence by day 12 to 15 in the lumen of the uterine glands.

Murray and Grifo (1976) reported evidence that gilts are incapable of secreting adult patterns of uterine specific proteins when treated with gonadal steroids until they reach about 140 days of age. However, they reported finding partial secretory patterns by 112 days of age. These findings indicated that the ability to secrete the complete pattern of uterine specific proteins comes with maturity.

IX. GnRH AND THE RELEASE OF LH

The role of LH and the hypothalamo-pituitary mechanism by which it is controlled has been further elucidated since its releasing hormone has been isolated and its chemical structure identified and synthesized. Matsuo et al. (1971a) determined the structure of porcine LH/FSH releasing hormone (GnRH) and subsequently synthesized the molecule in the laboratory (Matsuo et al., 1971b). Before the synthetic form of the releasing hormone was available, the only source had been hypothalmic extracts. Several reports have demonstrated that the synthetic form of the hormone is much more potent than the natural extracted releasing hormone (Matsuo et al., 1971b; Jaramillo Jaramillo et al., 1978).

X. LH LEVELS DURING THE ESTROUS CYCLE

During the estrous cycle of the pig and other mammals, it has been shown that the concentration of plasma LH reaches its peak value on day 1 of the cycle (Niswender et al., 1970; Rayford et al., 1971; Henricks et al., 1972). The peak which occurs at this time is often referred to as the "preovulatory LH surge." Niswender et al. (1970) and Henricks et al. (1972) have reported that peak LH concentrations in the pig range from 1.5 to 5.0 ng/ml with the higher values more common. Although these concentrations are significantly less than peak values of cows and sheep, LH values during other times of the pigs' estrous cycle are greater than those of sheep and cows (Niswender et al., 1970).

It has been suggested that there could be two possible ways by which the mechanism controlling the preovulatory LH surge operates.

Antunes-Rodriguez et al. (1966) postulated that the pituitary responsiveness to GnRH may be constant, and an increase of GnRH release may be responsible for the LH surge. Bogdanove (1964) postulated that there is a constant release of GnRH with increased pituitary sensitivity to GnRH with increased pituitary sensitivity to GnRH at estrus or both. Reeves et al. (1970a) showed that in sheep treated at diestrus with GnRH at various dose levels, a maximum response was found to occur which was not surpassed by higher doses of releasing hormone. The LH levels that resulted were not as high as those of the preovulatory surge and, therefore, demonstrated a possible difference in pituitary sensitivity to the releasing hormone.

In another experiment (Reeves et al., 1971b), it was reported that anestrous ewes pretreated with estradiol benzoate showed greater LH levels in response to GnRH treatment than ewes not primed with estrogens prior to treatment. The same workers showed greater LH concentration values in response to GnRH administration on day 1 of the estrous cycle of sheep than on any other day of the cycle, indicating greater pituitary responsiveness on day 1 (Reeves et al., 1971a). Aiyer and Fink (1974) reported that estradiol augmented the release of LH in response to GnRH in rats ovariectomized at diestrus, demonstrating increased pituitary sensitivity to GnRH. Aiyer et al. (1974) demonstrated a priming effect of releasing hormone in the rat which was augmented by estradiol administration. They showed that two successive doses of GnRH elicited LH responses such that the response to the second injection was greater than the first, but this response was less at metestrus and diestrus than it was at proestrus. These results indicated that the pituitary sensitivity

at proestrus depended upon the rise of endogenous estrogens at that particular time of the cycle. Arimura et al. (1972) reported a similar potentiating effect when single, large dosages of GnRH were administered to sheep. Symons et al. (1974) demonstrated a potentiating effect of GnRH on LH release when given in two injections three hours apart. In both experiments, the responses were dose dependent. Arimura et al. (1972) suggested the larger dose stayed in the blood long enough to sensitize the pituitary to the further release of LH, or the sensitization of the pituitary may have been indirectly due to a possible stimulation of ovarian estrogen.

Arimura and Schally (1970) demonstrated in rats that high levels of progesterone in the blood significantly suppressed the LH released in response to GnRH administration. Wright et al. (1978) reported a marked decrease in LH release in sheep previously treated with progesterone or a combination of progesterone and estrogen. It has been reported that the plasma LH values of the pig begin to rise slightly when plasma progesterone concentrations begin to fall and that peak LH levels are found when progesterone levels are minimal (Henricks et al., 1972).

XI. PITUITARY LH STORES

Experiments where GnRH has been administered by way of repeated injections or constant infusion have provided useful information regarding pituitary synthesis and storage of LH. Rippel et al. (1972) reported a depletion of pituitary LH in prepubertal ewes after injection of GnRH at six-hour intervals for three days. The depletion of pituitary LH was evident after the first maximal LH peak which occurred in response to

the initial injection. Chakraborty et al. (1972) showed that prepubertal gilts injected with GnRH at six-hour intervals for 96 hours responded with high peak LH levels after the first three injections, followed by decreased LH responses, which were consistent throughout the rest of the experiment. It was suggested that the decline in the LH response could have been due to a gradual decline in pituitary sensitivity to GnRH after repeated injections or a depletion in the pituitary LH stores occurred after the first few injections, with successive LH response levels related to the amount of LH synthesized in the six-hour time span between injections. In anestrous ewes, it has been demonstrated that it takes approximately 96 hours for the LH response to GnRH injection to reach the levels which follow initial treatment (Rippel et al., 1974). This indicated a refractory period might also exist in the anestrous ewe. This same response was reported by Symons et al. (1974).

GnRH infusion studies have been made in a number of species in order to determine the profile of the pituitary LH release pattern over time. Chakraborty et al. (1974) administered GnRH to anestrous ewes at a rate of 2.3 μg/hr and found peak LH levels occuring after three hours, with a subsequent decrease to pretreatment levels by 16 hours. Symons et al. (1974) reported a similar response in anestrous and cyclic ewes when GnRH was infused at a rate of 20 μg/hr for eight hours.

Bremner et al. (1976) found a different release profile that occurred when rams were infused with GnRH. Dose rates of 0.5 and 1.0 μg/min resulted in a biphasic pattern of LH release, with the second peak being greater than the first. Biphasic LH release patterns have also

been found to occur in the mature human male (Bremner and Paulsen, 1974; Beitins et al., 1977).

Hoff et al. (1977) demonstrated a biphasic release of LH in adult human females at all phases of the menstrual cycle, but the relative height of the second peak was found to be greatly influenced by ovarian steroid feedback. DeKretser et al. (1978) reported similar results, with the exception of a failure to find the biphasic response at the periovulatory period. TerHarr (1978) reported a biphasic LH release pattern in the guinea pig, with potentiation of the pituitary toward a greater LH concentration level at the second peak.

These and other studies have suggested that the pituitary of the mature animal in these species has two "pools" of LH. The second pool is supposedly released or synthesized when the pituitary is sufficiently stimulated with GnRH. Beitins et al. (1979) suggested that the first pool is stored in granules which lie close to the cell membrane for easy release from the gonadotrophin-producing cells, and the second pool is further inward and attach to microtubules prior to release.

XII. THE LH RELEASE PROFILE AS AN INDICATOR OF PITUITARY MATURITY

Reiter et al. (1976, 1977) demonstrated a difference between prepubertal and pubertal children in the ability to release LH when infused with GnRH. In both males and females, it was shown that pubertal children had a greater peak LH response than prepubertal children. More importantly, it was found that the LH response in immature (prepubertal) children was

of a monophasic nature, with a single peak, whereas the more mature (pubertal) children showed the biphasic response as found in adult humans (Bremner and Paulsen, 1974; Beitins et al., 1977; Hoff et al., 1977; DeKretser et al., 1978), rams (Bremner et al., 1976), and guinea pigs (TerHarr, 1978).

XIII. SUMMARY

It has been shown that many factors interact in the functioning of the reproductive system in swine, including external environmental influences, such as nutrition and social interaction, and several internal mechanisms. Although prepubertal gilts can be artificially stimulated to ovulate and show estrus, they fail to maintain pregnancy beyond the early stages. Several theories have evolved attempting to explain this phenomenon, although none have been proven conclusively.

Of all the attempts made using exogenous hormones to maintain pregnancy in stimulated gilts, HCG administration appears to hold the greatest promise of success. Reports indicating that the uterus is responsible for the failure of pregnancy maintenance, therefore, require further substantiation. Most of the work which has been done thus far appears to indicate that maintaining the integrity of the corpus luteum is of paramount importance. However, it is still unclear whether or not corpus luteum regression in prepubertal gilts is due to a lack of luteogrophic support or an imbalance between the luteotrophic and luteolytic mechanisms. Either case suggests an immaturity of the pituitary gland and/or hypothalamo-pituitary axis.

The report of Reiter et al. (1976, 1977), which demonstrated monophasic LH release patterns in prepubertal children vs. biphasic patterns in pubertal children, has proven useful in finding an indicator of pituitary maturity in the human. The question arises as to whether or not such a condition exists in other species.

The purpose of this experiment was to determine if there exists a difference in the LH release profile between prepubertal and mature cycling gilts under constant infusion of GnRH. This was done in an effort to provide evidence that if an immature pituitary exists in the prepubertal gilt, it may be in some way responsible for the inability of the animal to maintain pregnancy when puberty is induced artifically with exogenous hormone treatments.

CHAPTER III

MATERIALS AND METHODS

I. ANIMALS AND MANAGEMENT

This study was conducted in late spring and early summer of 1979. Two groups of crossbred gilts, taken from the University of Tennessee swine herd, were utilized in the experiment. The first group consisted of three immature gilts having an average age of 95 days and average body weight of 34 kg. The second group consisted of three mature cycling gilts having an average age of 12 months and average body weight of 145 kg.

The immature gilts were individually penned in metabolism crates which had moveable top and side boards for restraining purposes. The mature cycling gilts were individually penned in large metabolism stalls suitable for sows. Both groups were fed a calculated 16% crude protein grower-finishing diet consisting of ground corn and soybean meal. The immature gilts were fed the ration ad libitum, whereas the mature cycling gilts were fed at the rate of 2.72 kg/day. Water was provided at all times.

Estrus was detected in the mature cycling gilts using the hand pressure-immobility response method (Day, 1968) when they were in the presence of mature boars. Observations were also made for vulvular swelling and mounting on or by other gilts. Only those gilts consistently showing normal cycles of 21 $\pm$ 2 days, based on seven cycles, were used in the experiment.

II. CANNULATION

Indwelling cannulae were implanted unilaterally in one jugular vein of each gilt in the experiment. The cannulae were used for infusion of GnRH and for blood sampling.

Anesthesia was induced in the prepubertal gilts using pentobarbital (Nembutal; Abbott, N. Chicago, Illinois), administered intravenously in an ear vein. Thiamyl sodium (Surital; Park, Davis & Co., Detroit, Michigan), given intravenously in an ear vein, was used to induce anesthesia in the mature cycling gilts. In both groups, the surgical plane of anesthesia was maintained with Halothane (Ayerst, New York, New York), administered through intranasal tubes.

The cannulae which were implanted into the jugular veins of the immature gilts were made of polyethylene tubing, 1.270 mm ID x 2.286 mm OD. These were held in place by loops of suture material sewn into the muscle and fasciae internally and to the skin externally. The tubing was covered on the outside with a band of adhesive tape held loosely around the neck. A blunt-edged, 18-gauge needle was held firmly within the outside end of each cannula and capped off with a male Luer-Lok cap (Becton-Dickenson, Rutherford, New Jersey). The cannulae were flushed daily with heparinized saline during a recovery period of three days.

The cannulae used in the mature cycling gilts were made of silastic tubing (Dow-Corning, Midland, Michigan), 1.016 mm ID x 2.159 mm OD, and were held in place by feeding them through a cuff of silastic tubing, 1.574 mm ID x 2.159 mm OD, that was 31.75 mm in length. The cuff was sutured in the muscle and fasciae on either side of the jugular vein.

These cannulae were exteriorized through the back of the neck after passing dorsolaterally under the skin. Excess tubing was kept in a pouch of cotton denim material 63.50 mm x 63.50 mm, which was sutured to the skin. The ends of the cannulae were capped in the same manner as for the immature gilts, and heparinized saline was flushed through daily. These gilts were also given a three-day surgery recovery period.

III. DOSING AND BLOOD SAMPLING

Ten-ml blood samples were taken at 15-minute intervals over a period of eight hours during the experiment, for a total of 32 samples. These were placed into heparinized tubes and centrifuged for 20 minutes at 2500 rpm and 650 x g. Plasma samples were extracted and frozen at -20°C until assayed for LH. The red cells of the immature gilts were resuspended in physiological saline and returned to the animals.

A new synthetic analogue of GnRH was used in the experiment (American Hoechst product no. HOE 766, American Hoechst, Somerville, New Jersey). This was administered to both groups in pulses given at regular six-minute intervals at a dose rate of 2.3 μg/hr regardless of body weight for a total of six hours. Dosing began after the first hour baseline period and ended after the seventh hour. This was done at about day 17 of the estrous cycle of the mature animals to ensure against high levels of progesterone that would prevent an LH release in response to the releasing hormone. Time was not a critical factor for the immature gilts.

IV. RADIOIMMUNOASSAY OF LH

Assay for plasma LH values was done using a double antibody human LH radioimmunoassay system which is available in kit form (Radioassay Systems, Inc., Carson, California, catalog no. 124). Individual kits were used for each gilt, and interassay variations were determined between consecutive sets of two kits. Each kit had a capacity of analyzing 100 tubes; 18 were reserved to construct the standard curve, and 64 were used to measure the 32 samples in duplicate. The 18 tubes which remained in each kit were used in determining interassay variations. Since two kits were used on any one day, nine similar samples chosen at random from the series taken from each gilt were assayed in both of the two kits used on that day. The reference numbers used for the gilts were 1, 2, and 3 for the immatures and 4, 5, and 6 for the mature animals. Gilts 1 and 2 were assayed with kits 1 and 2, and the other followed likewise.

V. RADIOIMMUNOASSAY VALIDATION

A partial validation of the kit was done to show that porcine LH could be measured. The validation procedure consisted of two parts. The first part was an immunological validation which utilized plasma samples taken from mature cycling gilts injected with estradiol benzoate eight hours prior to blood sampling in an effort to induce the pituitary release of LH (Dial, 1978). These samples were analyzed along with samples from non-estradiol treated gilts in an effort to determine if the human

LH kit would give any reliable indication of LH concentrations in porcine plasma.

The second part of the validation procedure was a biological validation which utilized plasma samples taken from a mature cycling gilt at day 17 of the estrous cycle. Blood samples were taken every ten minutes for four hours. The first hour was a control, or baseline, period, during which physiological saline was infused intravenously through an indwelling cannula. After the baseline period, a massive dose of 15 μg of GnRH was infused over the next three hours. The purpose of this part of the validation procedures was to determine if the LH kit would show a cause-effect response with LH being released under GnRH administration.

Both validation procedures examined parallelism trials for the human LH kit to determine if there were proportionate increases or decreases in the assumed LH measurements when plasma sample volumes were correspondingly varied. The validation procedures also examined percent recovery trials for the human LH kit. Percent recovery indicates the percentage of a known concentration of hormone standard that is left when incubation reactions take place with a constant volume of sample plasma of known concentration competing with it. This step is intended to show that the LH in the sample can and does compete with the hormone standard for the same binding sites on the antibody to the hormone standard.

VI. STATISTICAL ANALYSIS

Differences between gilts within groups concerning baseline levels of LH and mean response levels of LH during GnRH infusion were determined

using one-way analysis of variance procedures. Where differences arose, Duncan's multiple range test was used to determine which means were different between gilts within groups. Differences between the two groups' mean baseline LH levels, overall mean LH response levels to GnRH infusion, and the change in LH from mean baseline to peak LH levels were determined using two-tailed t-tests.

Coefficients of variation were calculated for similar plasma samples assayed in each of two assay kits used on any one day. Mean values, standard deviations, and ranges were determined for these, and overall means, ranges, and standard deviations were calculated from the composite of data. Duncan's multiple range test was used to determine whether there were differences between the mean values of the coefficients of variation for each pair of kits.

CHAPTER IV

RESULTS AND DISCUSSION

I. RADIOIMMUNOASSAY VALIDATION

The results for the immunological and biological phases of the radioimmunoassay validation procedures of recovery and parallelism are given in Tables I and II respectively.

Specificity

The radioimmunoassay kit antiserum used in this study was specific for human LH, but the validation procedures that were followed showed porcine LH could be measured reliably, as shown in the following account.

Cross-Reactivity

The antibody used has been found to have 100% cross-reactivity with HCG, 15% with human thyroid stimulating hormone (hTSH), 1% with human follicle stimulating hormone (hFSH), and 1% with human growth hormone (hGH).

Sensitivity

The sensitivity of the standard curve was found to be 0.5 to 1.0 mI.U./ml or 0.06 ng/ml to 0.12 ng/ml when units are converted to those used in the literature for the pig.

Recovery

In the recovery procedure for the immunological phase, percent recovery was considered good, with percentages of 104, 87, and 99 for the 2.5, 5.0, and 50.0 mI.U./ml standards respectively when reacted with 0.1 ml

TABLE I

RECOVERY

Part	Contribution from Standard (mI.U./ml)	Contribution from Pooled Plasma* (mI.U./ml)	Amount Recovered (mI.U./ml)	Amount Expected	% Recovery ($\frac{\text{Recovered}}{\text{Expected}}$)
I	2.5	2.2	4.9	4.7	104
	5.0	2.2	6.3	7.2	87
	50.0	2.2	52.0	52.2	99
II	2.5	9.8	12.3	12.3	100
	5.0	9.8	14.3	14.8	97
	50.0	9.8	70.0	59.8	116

*Part I used 0.1-ml known sample volume; Part II used 0.3-ml known sample volume.

TABLE II

PARALLELISM

Part	Assayed (ml)	Observed Concentration (mI.U./ml)	Expected Concentration (mI.U./ml)	% Difference from Expected
I	0.10	5.60	---	---
	0.15	5.90	8.40	29.76
	0.20	10.00	11.20	10.71
	0.30	12.10	16.80	27.97
II	0.10	1.90	---	---
	0.15	3.20	2.85	12.28
	0.20	4.40	3.80	15.78
	0.30	6.60	5.70	15.78

of known plasma sample. The corresponding values given for the biological phase were 100, 97, and 116% recovery. These results indicated that in the plasma samples of these gilts, there was evidence of the existence of a hormone which was assumed to be porcine LH, and this competed with the human LH standards for binding sites on the antibody to human LH. The fact that the percent recovery values in the biological phase of validation showed no difference from those in the immunological validation provided further evidence of the measurement of porcine LH, since a larger volume (0.3 ml) of known plasma sample was used in the former case. The result of 104% recovery for the 2.5 mI.U./ml standard in the immunological phase of validation and 100% recovery for the same standard concentration in the biological phase of validation indicate that the human LH antibody may have had a strong affinity for porcine LH, with a one-to-one competition for the antibody at this standard concentration.

Parallelism

The results for the parallelism procedures in Parts I and II demonstrated increasing concentrations of the assumed LH being measured in relative proportion to increasing volumes of the pooled sample. The percentages of the amount of difference from expected values were considered reasonable and ranged from 10.7 to 27.9% in Part I and from 12.2 to 15.7% in Part II.

Interassay Variation

Results for the modified interassay variation calculations are presented in Table III. The overall coefficient of variation ranged from 0.00 to 16.34, with an overall mean of 5.26 and standard deviation of 3.44.

TABLE III

INTERASSAY VARIATIONS

Kits Compared	Coefficients of Variation		
	Range	Mean	Standard Deviation
1 and 2	0.24 to 9.53	4.34[a]	2.76
3 and 4	0.00 to 9.93	4.69[a]	2.82
5 and 6	0.75 to 16.34	6.75[b]	4.21
Overall	0.00 to 16.34	5.26	3.44

[a,b]Values in mean column with different superscripts are different ($P < .05$).

Duncan's multiple range test indicated that the mean coefficient of variation calculated for kits 5 and 6 (6.75) were significantly ($P < .05$) different from those found for kits 1 and 2 (4.34) and kits 3 and 4 (4.69), although this difference was rather small.

LH values extrapolated from standard curves and converted from mI.U./ml values to ng/ml values showed LH concentrations ranging from 0.242 to 0.696 ng/ml. These values fall considerably below the values that have been reported for the peak LH values of the preovulatory surge (Niswender et al., 1970; Henricks et al., 1972). Chakraborty et al. (1972) reported peak LH levels of 6.4 ng/ml in response to repeated dosages of 25 µg of GnRH. Therefore, it appears that the dose of GnRH used in this experiment was insufficient to ellicit LH release in quantities like those of the preovulatory surge. The values for LH found in this experiment come closer to those reported by Henricks et al. (1972) for times other than estrus, but conflict with those reported by Niswender et al. (1970) for times other than estrus.

II. LH RELEASE PATTERNS

The LH release patterns during the experiment for each individual prepubertal gilt are illustrated in Figures 1 through 3, and those of the mature cycling gilt are illustrated in Figures 4 through 6. The profiles for the individual group averages are plotted together in Figure 7.

Analysis of variance determined that there was no significant difference ($P > .05$) for the mean baseline LH values between the prepubertal gilts, but it was found that gilt number 4 of the mature group had a significantly different ($P < .05$) mean baseline LH concentration. This had

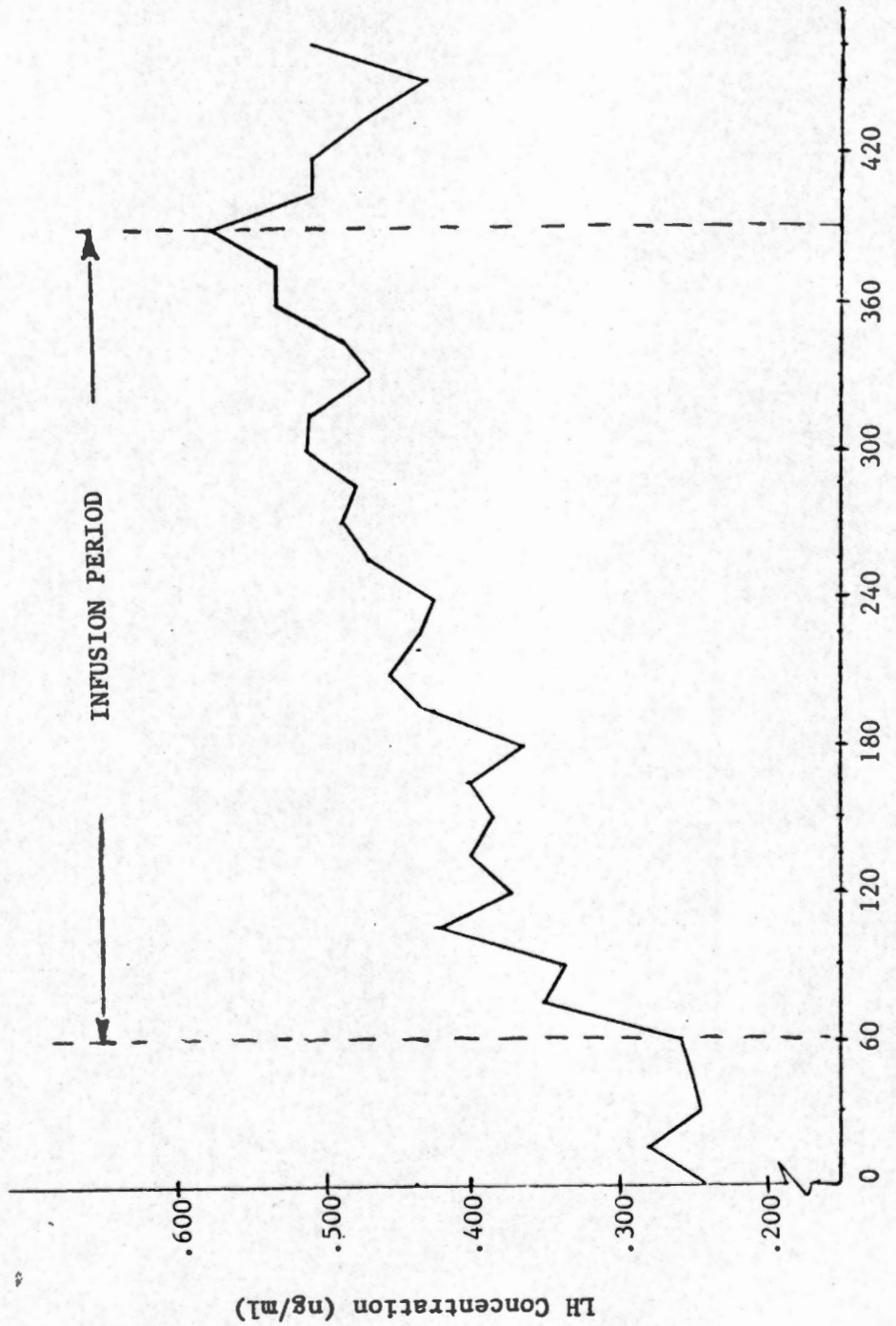


Figure 1. LH Profile for Pig Number 1 (Prepubertal).

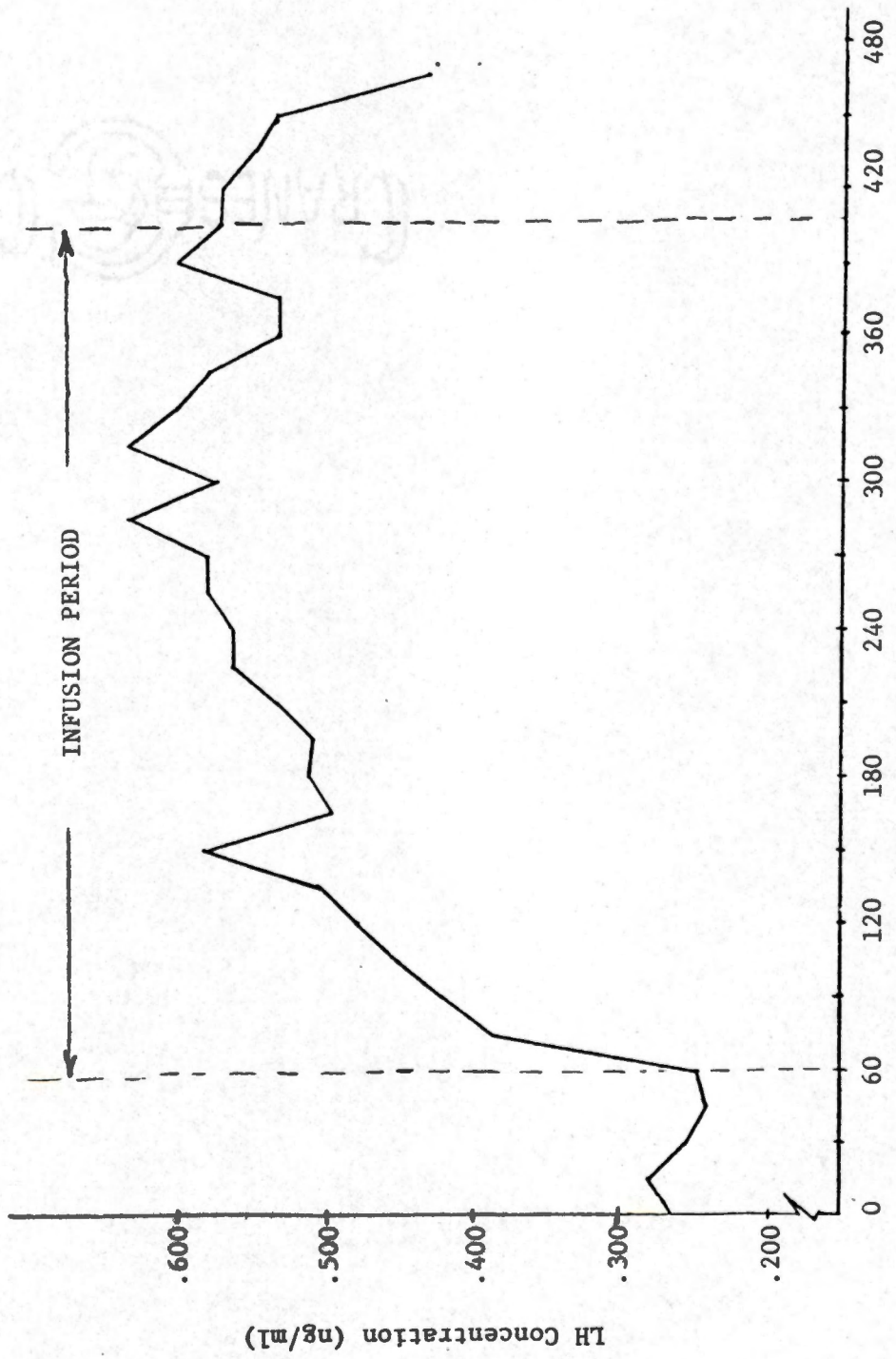


Figure 2. LH Profile for Pig Number 2 (Prepubertal).

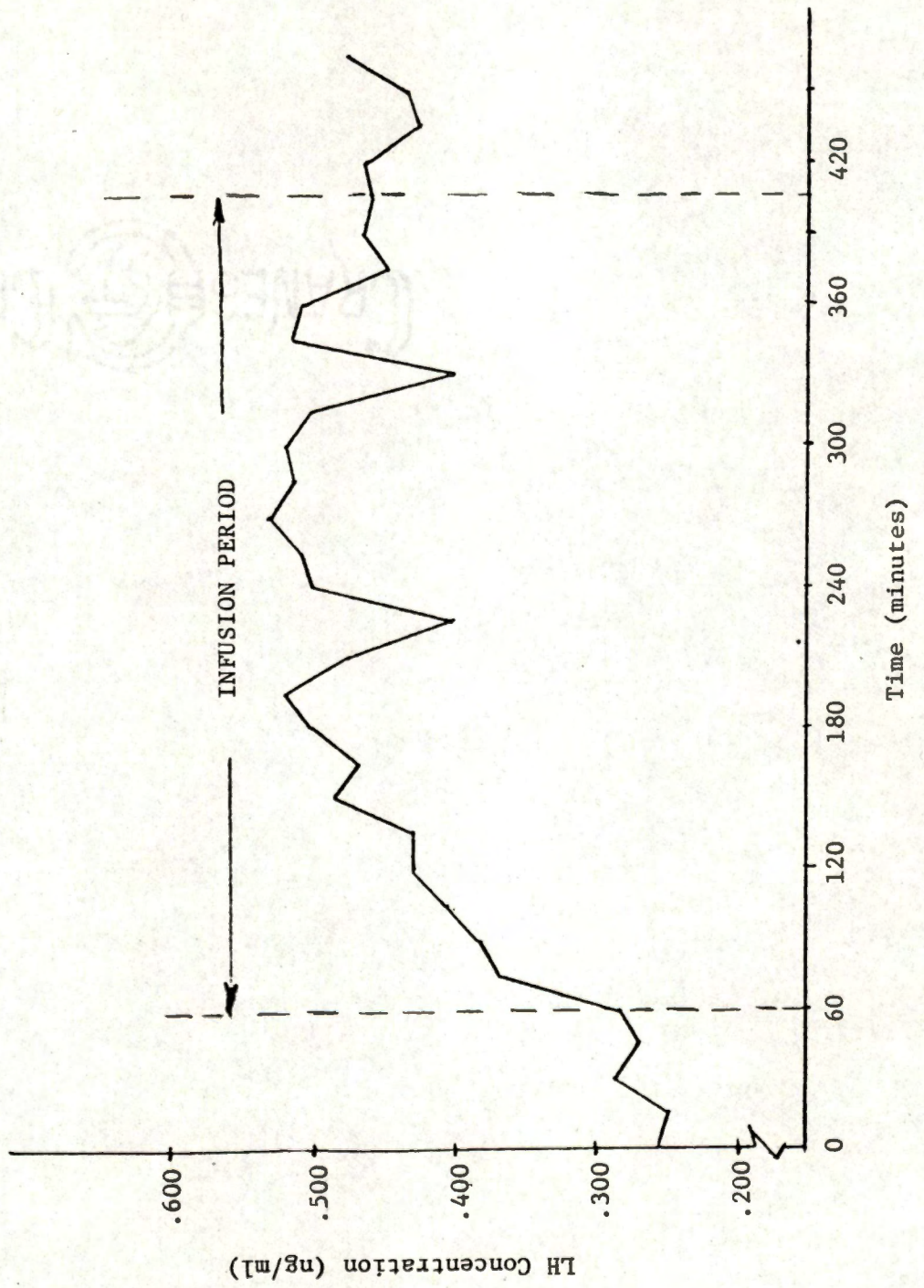


Figure 3. LH Profile for Pig Number 3 (Prepubertal).

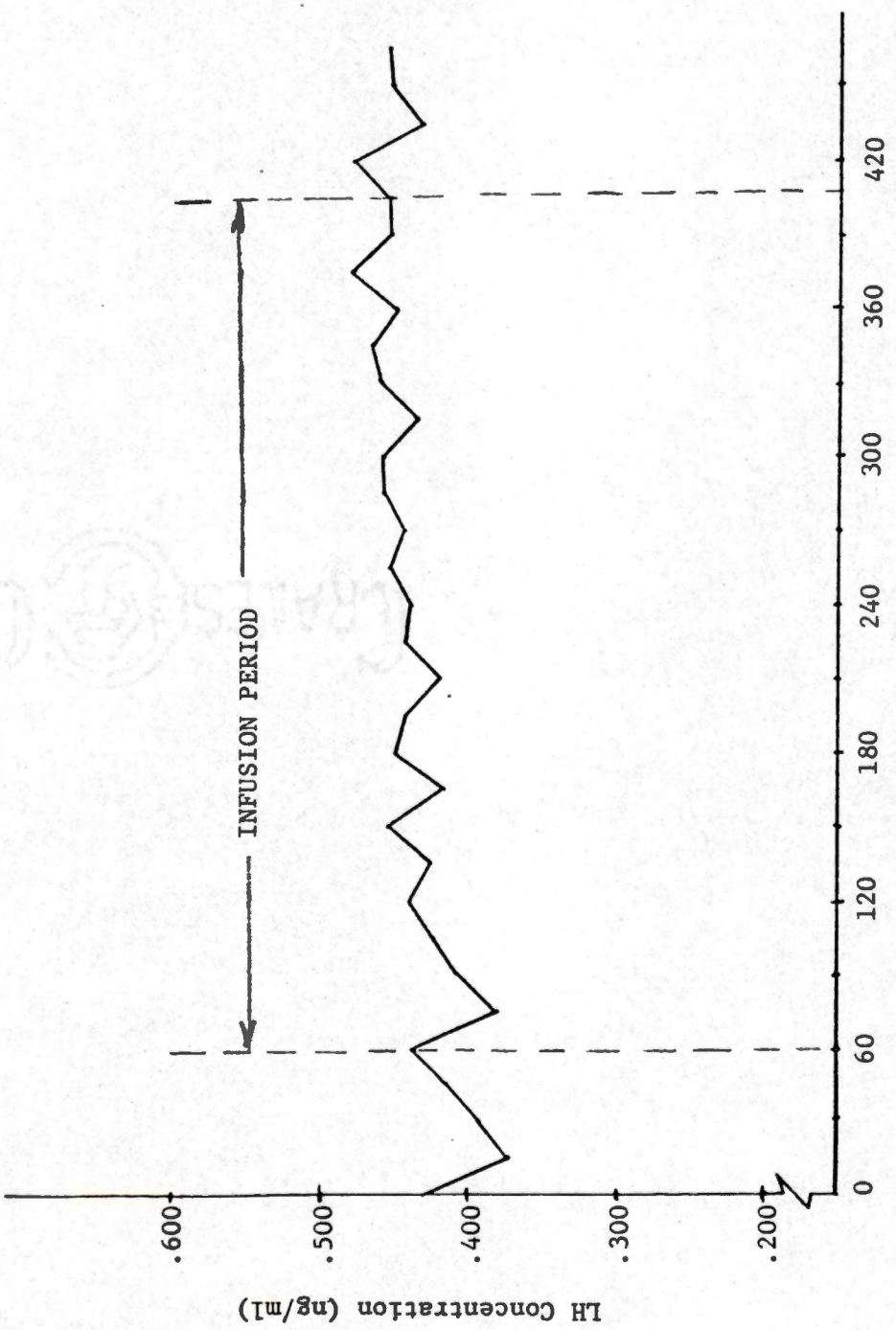


Figure 4. LH Profile for Pig Number 4 (Mature Cycling).

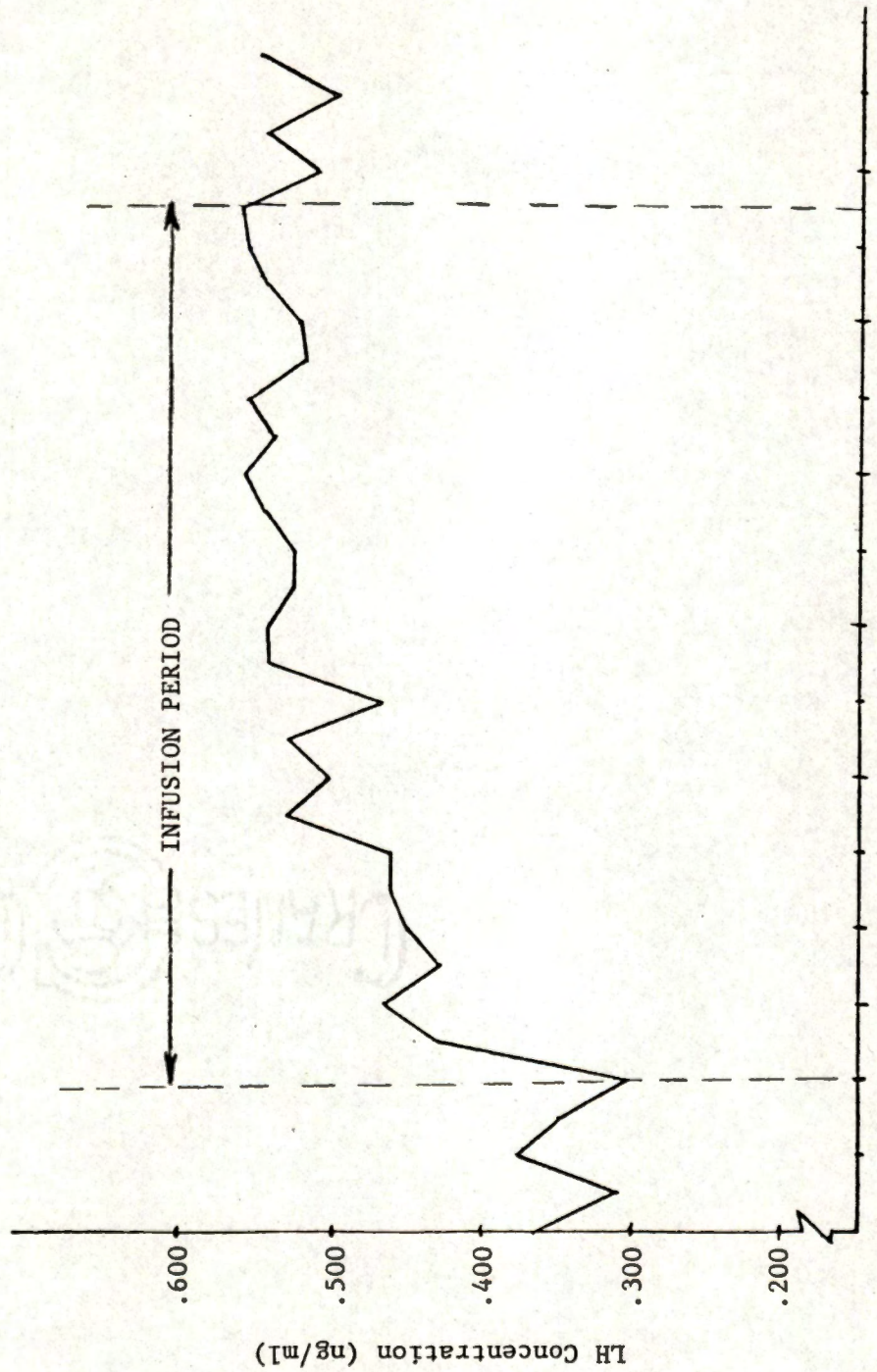


Figure 5. LH Profile for Pig Number 5 (Mature Cycling).

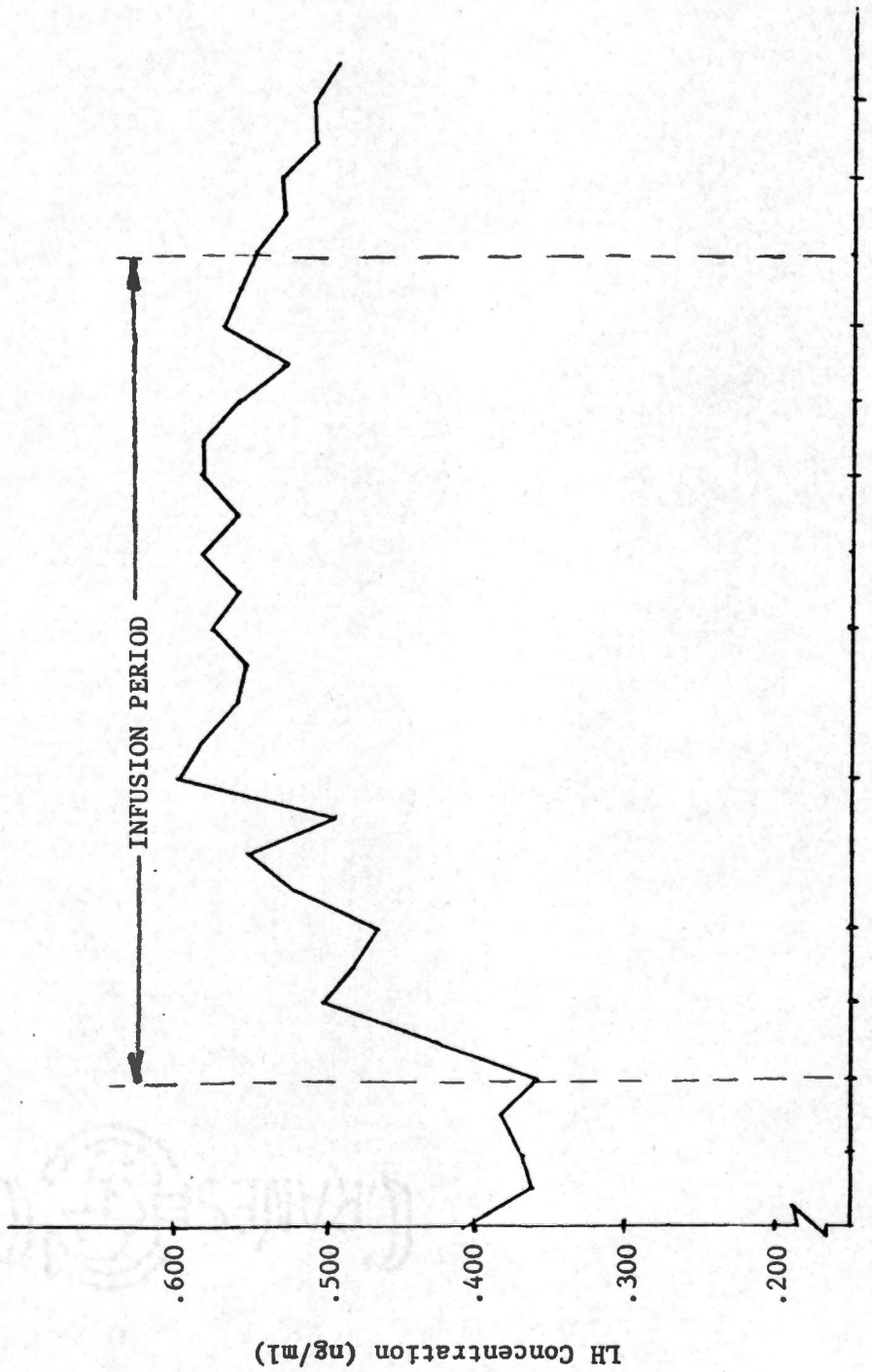


Figure 6. LH Profile for Pig Number 6 (Mature Cycling).

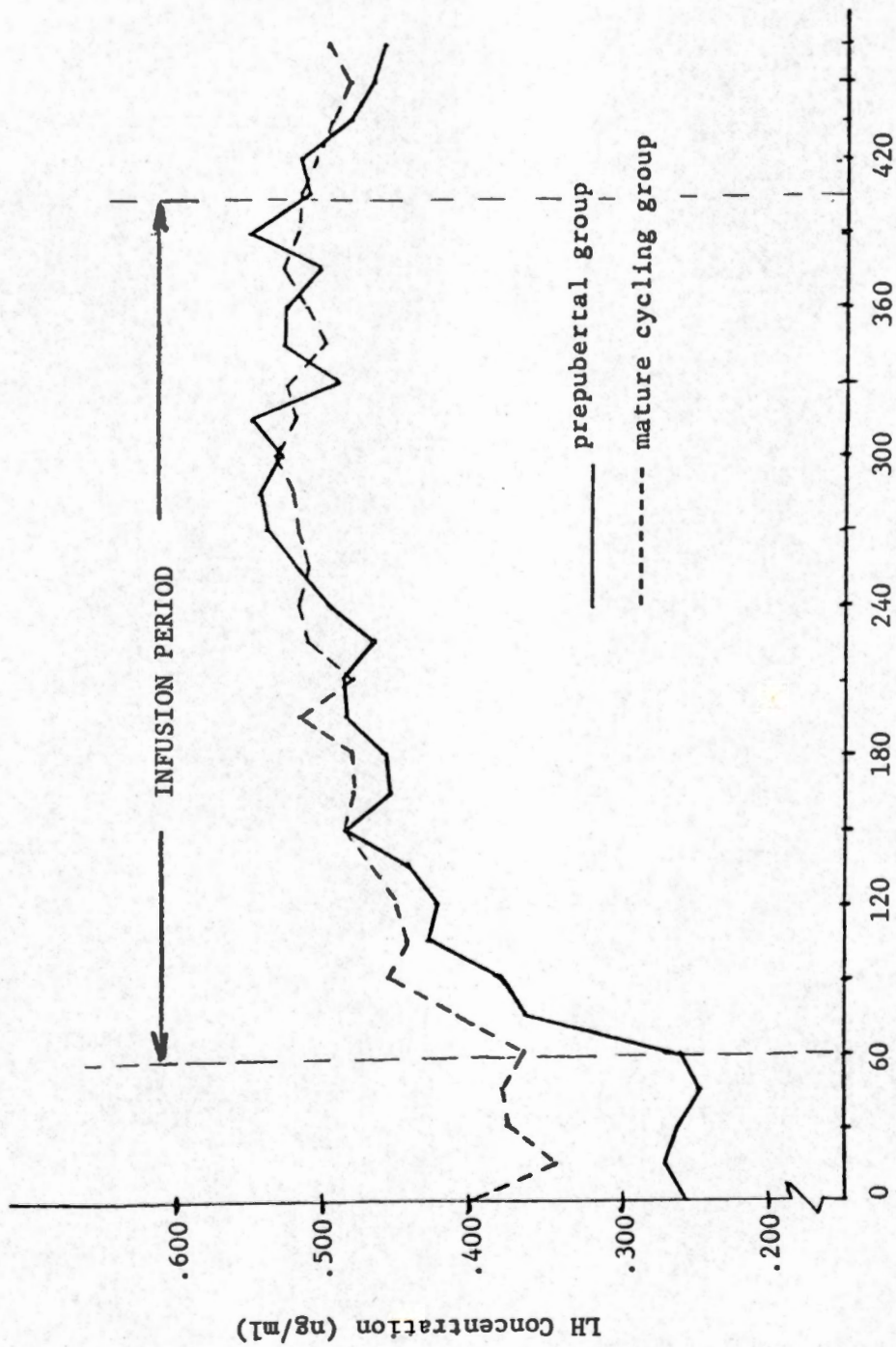


Figure 7. Mean LH Profile for Prepubertal and Mature Cycling Gilts.

no effect on the t-test, which showed that there was a significant difference ($P < .05$) between the mean baseline LH levels for the two groups, with the prepubertal gilts showing a lower mean baseline LH concentration of 0.26 ng/ml compared to 0.37 ng/ml or 0.35 ng/ml in the mature gilts with the different gilt included and excluded, respectively.

It was found that at least one gilt in each group was significantly different ($P < .05$) from the other gilts in her respective group in terms of mean LH response values under GnRH infusion. Regardless of whether or not within-group gilts were eliminated from the analysis, the t-test showed no significant difference ($P > .05$) between groups for the mean LH response during GnRH infusion.

The overall mean change from mean baseline to peak LH values were calculated for each group, and it was determined that there was no significant difference ($P > .05$) between groups for this parameter. This was so regardless of whether or not gilts with different within-group peak values were included in the analysis.

The results demonstrate that the major difference found between the LH release profile of the prepubertal gilts and the mature cycling gilts was that the pretreatment baseline levels of the immature gilts were significantly less ($P < .05$) than those of the mature animals. Although studies such as this one have not been published for swine, these results might have been anticipated. Earlier reports in swine (Hollandbeck et al., 1956; Parlow et al., 1964), rats (Goldman et al., 1971), and bulls (Macmillan and Hafs, 1966) demonstrated that the LH content of the pituitary is considerably higher before puberty than after puberty, but the levels of LH in the blood are considerably low until after the onset of

puberty. Ramirez and Sawyer (1966) showed that in the rat blood, levels of gonadal hormones are higher after puberty as a result of an increased release of gonadotrophins, which is, in turn, due to the maturation of the hypothalmus which releases the gonadotrophin releasing hormone.

The lack of a significant difference ($P > .05$) between groups for the mean LH response values during GnRH infusion and also for the change from baseline to peak levels of LH can be explained as partly due to an insufficient number of animals per group and a dose effect. For the case of the change from baseline to peak LH levels in particular, it was found that the mean change in LH concentration was 0.32 ng/ml and 0.17 ng/ml for the prepubertal vs. the mature cycling gilts respectively. Although these values appear to be very different, the t-test showed them not to be significantly different. The reason for this outcome was that the degrees of freedom allowed for so few a number of gilts was such that the tabulated value of "t" fell far above the test statistic for "t." This would not have been the case had even one more experimental animal been used per group.

Since no significant differences ($P > .05$) were found between groups for the mean LH response levels during GnRH infusion and since the immature gilts displayed a greater, although nonsignificant ($P > .05$), change in LH values from baseline to peak levels than the mature cycling gilts, it was concluded that this was due to a greater dosage of GnRH administered to the immature gilts in terms of micrograms of GnRH per kilogram of body weight. Since the immature gilts received 2.3 µg/34 kg/hr or 0.0676 µg/kg/hr and the mature cycling gilts received 2.3 µg/145 kg/hr or 0.0158 µg/kg/hr, the immature gilts received a dose rate that was the

equivalent of 4.27 times that of the mature cycling gilts. Rippel et al., (1972) and Rippel et al. (1974) demonstrated pituitary refractoriness to GnRH when low dosages were frequently injected into sheep. Chakraborty et al. (1972) reported similar refractoriness in prepubertal gilts. However, Arimura et al. (1972) and Symons et al. (1974) demonstrated that large dosages of GnRH injected into sheep had the effect of producing a biphasic pattern of LH release where the second peak was of greater magnitude than the first. From this information and from the results of this experiment, it is probable that the greater, although insignificant, change in LH levels from baseline to peak for the prepubertal gilts was due to this dose effect.

Inspection of the LH release profile in Figures 1 through 7 for the gilts in the study shows no clear-cut difference between the prepubertal and pubertal gilts for the pattern of LH release under GnRH infusion. No monophasic and biphasic LH release patterns were found as those described in prepubertal and pubertal children, respectively, by Reiter et al. (1976, 1977). Gilt 3 of the immature group showed a precipitous, although temporary, decline occurring twice in the LH release profile at sample 16 taken at 225 minutes into the experiment and sample 23 taken at 330 minutes. Gilt 1 appeared to follow this trend as well, but to a lesser degree. Although the numbers of animals used in the experiment prohibit any definite speculation about this, it seems possible that a temporary pituitary refractoriness may have occurred. A similar corresponding decline was seen in gilt 5 and also in gilt 6, although less markedly, which occurred at sample 15 taken at 205 minutes into the experiment. Again, no concrete speculations could be made. The very minimal LH response to GnRH infusion

in gilt 4 was probably the result of the stress of transport, which could have upset the estrous cycle. It is suggested that the lack of a biphasic LH response to GnRH infusion in the mature cycling gilts was due to the fact that the dose used was insufficient to cause potentiation of the pituitary gland to release or synthesize the greater amounts of LH from the second gonadotrophic pool of LH as described in previous studies (Bremner et al., 1976; Bremner and Paulsen, 1974; Beitins et al., 1977; Hoff et al., 1977; DeKretser et al., 1978; TerHaar, 1978; Reiter et al., 1976, 1977).

CHAPTER V

SUMMARY

An attempt was made in this study to establish an indicator of the maturity of the pituitary and/or hypothalamo-pituitary axis by comparing the LH release profile under continuous infusion of GnRH in the prepubertal and mature cycling gilt. This was done in an effort to show whether or not an immature pituitary gland could be responsible for failure to maintain pregnancy in the prepubertal gilt when puberty is induced.

The experiment consisted of three prepubertal gilts in one group and three cyclic gilts in a second group. GnRH was administered at a dose rate of 2.3 μg/hr given in pulses over six hours, with blood sampling taking place before, during, and after infusion. Plasma LH values were determined with a human LH radioimmunoassay kit which was shown to be able to detect porcine LH with some reliability.

Plasma LH values detected by this kit were below those levels found by some workers for the preovulatory LH surge but were within the range of values which have been reported for times other than estrus. The results showed a significant ($P < .05$) difference in the mean baseline LH values between groups and no significant ($P > .05$) difference between groups for mean LH response levels during GnRH infusion. There appears to be a greater change from mean baseline to peak LH levels in the prepubertal gilts, but the increase was not significant ($P > .05$). It was concluded that a single-peaked or monophasic LH release profile vs. a double-peaked or biphasic LH release profile was not evident in the immature and mature gilts respectively as noted in other species. This

appears to have been due to an insufficient dose rate of the releasing hormone (GnRH).

LIST OF REFERENCES

LIST OF REFERENCES

Aiyer, M. S., S. A. Chiappa, and G. Fink. 1974. A priming effect of luteinizing hormone releasing factor on the anterior pituitary gland in the female rat. J. Endocrinol. 62:573.

Aiyer, M. S. and G. Fink. 1974. The role of sex steroid hormones in modulating the responsiveness of the anterior pituitary gland to luteinizing hormone releasing factor in the female rat. J. Endocrinol. 62:553.

Anderson, L. L., F. C. Neal, and R. M. Melampy. 1962. Hysterectomy and ovarian function in beef heifers. Amer. J. Vet. Res. 23:794.

Anderson, L. L., K. P. Bland, and R. M. Melampy. 1969. Comparative aspects of uterine-luteal relationships. Recent Prog. Hormone Res. 25:57.

Antunes-Rodrigues, J., A. P. S. Dhariwal, and S. M. McCann. 1966. Effect of purified luteinizing hormone-releasing factor (LH-RF) on plasma LH activity at various stages of the estrous cycle of the rat. Proc. Soc. Exp. Biol. Med. 122:1001.

Arimura, A. and A. V. Schally. 1970. Progesterone suppression of LH-releasing hormone-induced stimulation of LH release in rats. Endocrinol. 87:653.

Arimura, A., L. Debeljuk, H. Matsuo, and A. B. Schally. 1972. Release of luteinizing hormone by synthetic LH-releasing hormone in the ewe and ram. Proc. Soc. Exp. Biol. Med. 139:851.

Baker, R. D. and E. G. Coggins. 1968. Control of ovulation rate and fertilization in prepubertal gilts. J. Anim. Sci. 27:1607.

Beitins, I. Z., M. L. Dufau, K. O'Loughlin, K. J. Catt, and J. W. McArthur. 1977. Analysis of biological and immunological activities in the two pools of LH released during constant infusion of luteinizing hormone-releasing hormone (LHRH) in men. J. Clin. Endocrinol. Metab. 45:605.

Bogdanove, E. M. 1964. The role of the brain in the regulation of pituitary gonadotropin secretion. Vitamins & Hormones. 22:205.

Braithwaite, S. S. and J. Jarabak. 1975. Studies on 15-hydroxyprostaglandin dehydrogenase from human placenta. Purification and partial characterization. J. Biol. Chem. 250:2315.

Bremner, W. J. and C. A. Paulsen. 1974. Two pools of luteinizing hormone in the human pituitary: evidence from constant administration of luteinizing hormone releasing hormone. J. Clin. Endocrinol. Metab. 39:811.

Bremner, W. J., J. K. Findlay, I. A. Cumming, B. Hudson, and M. DeKretser. 1976. Pituitary-testicular response in rams to prolonged infusions of luteinizing hormone-releasing hormone (LHRH). Biol. Reprod. 15:141.

Brooks, P. H. and D. J. A. Cole. 1970. The effect of the presence of a boar on the attainment of puberty in gilts. J. Reprod. Fertil. 22:435.

Cavazos, L. F., L. L. Anderson, W. D. Belt, D. M. Henricks, R. R. Kraeling, and R. M. Melampy. 1969. Fine structure and progesterone levels in the corpus luteum of the pig during the estrous cycle. Biol. Reprod. 1:89.

Casida, L. E. 1934. Ovarian stimulation in immature farm animals. Proc. Am. Soc. An. Prod. p. 144.

Casida, L. L. 1935. Prepubertal development of the pig ovary and its relation to stimulation with gonadotrophic hormones. Anat. Rec. 61:389.

Chakraborty, P. K., J. J. Reeves, A. Arimura, and A. V. Schally. 1973. Serum LH levels in prepubertal female pigs chronically treated with synthetic luteinizing hormone-releasing hormone/follice stimulating hormone (LH-RH/FSH-RH). Endocrinol. 92:55.

Chen, T. T. and F. W. Bazer. 1973. Effect of antiserum to porcine fraction IV uterine protein on the conceptus. J. Anim. Sci. 37:304 (Abstr.).

Chen, T. T., F. W. Bazer, B. Gebhardt, and R. M. Roberts. 1975. Synthesis and movement of porcine purple uterine protein. J. Anim. Sci. 40:170 (Abstr.).

Christensen, R. K. and B. N. Day. 1972. Luteolytic effects of endometrial extracts in the pig. J. Anim. Sci. 34:620.

Chu, J. P., C. C. Lee, and S. S. You. 1945. Functional relation between the uterus and corpus luteum. J. Endocrinol. 4:392.

Cunningham, P. J., C. H. Naber, D. R. Zimmerman, and E. R. Peo, Jr. 1974. Influence of nutritional regime on age at puberty in gilts. J. Anim. Sci. 39:63.

Day, B. N., L. L. Anderson, M. A. Emmerson, L. N. Hazel, and R. M. Melampy. 1959. Effects of estrogen and progesterone on early embryonic mortality in ovariectomized gilts. J. Anim. Sci. 18:607.

Day, B. N. 1968. Reproduction of swine. In: E. S. E. Hafez (Ed.), Reproduction in Farm Animals. Lea and Febiger, Philadelphia, PA.

DeKretser, D. M., H. G. Burger, and R. Dumpys. 1978. Patterns of serum LH and FSH in response to 4-hour infusions of luteinizing releasing hormone in normal women during menstrual cycle, on oral contraceptives, and in postmenopausal state. J. Clin. Endocrinol. Metab. 46:227.

Dhindsa, D. S. and P. J. Dziuk. 1968. Effect of pregnancy in the pig after killing embryos or fetuses in one uterine horn in early gestation. J. Anim. Sci. 27:122.

Dial, G. D., O. K. Dial, and D. J. Dziuk. 1978. Maturation of the LH response of the prepubertal gilt to pregnant mare's serum gonadotropin (PMSG) and estradiol benzoate (E2b). Biol. Reprod. 18 Suppl. 1:34a (Abstr.).

Diehl, J. R. and B. N. Day. 1974. Effect of prostaglandin F2α on luteal function in swine. J. Anim. Sci. 39:392.

Diehl, J. R., R. A. Goodke, D. B. Killian, and B. N. Day. 1974. Induction of parturition in swine with prostaglandin F2α. J. Anim. Sci. 38:1229.

Douglas, R. H. and O. J. Ginther. 1972. Effect of prostaglandin F2α on length of diestrus in mares. Prostaglandins. 2:265.

Douglas, R. H. and O. J. Ginther. 1973. Luteolysis following a single injection of prostaglandin F2α in sheep. J. Anim. Sci. 37:990.

Duncan, G. W., A. M. Bowerman, W. R. Hearn, and R. M. Melampy. 1960. In vitro synthesis of progesterone by swine corpora lutea. Proc. Soc. Exp. Biol. Med. 104:17.

Dziuk, P. J. and R. D. Baker. 1962. Induction and control of ovulation in swine. J. Anim. Sci. 21:697.

Dziuk, P. J. and G. D. Gehlbach. 1966. Induction of ovulation in the immature gilt. J. Anim. Sci. 25:410.

Friend, D. W. 1977. Effect of dietary energy and protein on age and weight at puberty in gilts. J. Anim. Sci. 44:601.

Gardner, M. L., N. L. First, and L. E. Casida. 1963. Effect of exogenous estrogens on corpus luteum maintenance in gilts. J. Anim. Sci. 22:132.

Gentry, B. E., L. L. Anderson, and R. M. Melampy. 1973. Exogenous progesterone and estradiol benzoate on early embryonic survival in the pig. J. Anim. Sci. 37:722.

Gleeson, A. R. and G. D. Thorburn. 1973. Plasma progesterone and prostaglandin F concentrations in the cyclic sow. J. Reprod. Fertil. 32:343 (Abstr.).

Goldman, B. D., Y. R. Brazie, I. A. Kamberi, and J. C. Porter. 1971. Serum gonadotropin levels in intact and castrated neonatal rats. Endocrinol. 88:771.

Gomes, W. R., R. C. Herschler, and R. E. Erb. 1965. Progesterone levels in ovarian venous effluent of the nonpregnant sow. J. Anim. Sci. 24:722.

Goode, L., A. C. Warnick, and H. D. Wallace. 1960. Effects of energy level on reproductive performance of gilts. J. Anim. Sci. 19:643 (Abstr.).

Guthrie, H. D. 1977. Induction of ovulation and fertility in prepubertal gilts. J. Anim. Sci. 45:1360.

Haines, C. E., A. C. Warnick, H. D. Wallace, and G. M. Edwards. 1957. The effect of level of feeding and progesterone injections on reproductive performance in gilts. J. Anim. Sci. 16:1099 (Abstr.).

Hallford, D. M., R. P. Wetteman, E. J. Turman, and I. T. Omtvedt. 1975. Luteal function in gilts after prostaglandin F2α. J. Anim. Sci. 41:706.

Henricks, D. M., H. D. Guthrie, and D. L. Handlin. 1972. Plasma estrogen, progesterone, and luteinizing hormone levels during the estrous cycle in pigs. Biol. Reprod. 6:210.

Hoff, J. D., B. L. Lasely, C. F. Wang, and S. S. C. Yen. 1971. The two pools of pituitary gonadotropin: regulation during the menstrual cycle. J. Clin. Endocrinol. Metab. 44:302.

Hollandbeck, R., B. Baker, Jr., H. W. Horton, and A. V. Nalbandov. 1956. Gonadotrophic hormone content of swine pituitary glands in relation to age. J. Anim. Sci. 15:418.

Jaraback, J. 1972. Human placental 15-hydroxyprostaglandin dehydrogenase. Proc. Nat. Acad. Sci., USA. 69:553.

Jaramillo Jaramillo, C., A. Charro-Salgado, V. Perez-Infante, G. Lopez DelCampo, J. Botella-Llusia, D. H. Coy, and A. V. Schally. 1978. Clinical studies with D-Trp -LH-RH in anovulatory women. Fert. and Steril. 29:418.

Karsch, F. J., J. F. Roche, J. W. Noveroske, D. L. Foster, H. W. Norton, and A. V. Nalbandov. 1971. Prolonged maintenance of corpus luteum of the ewe by continuous infusion of luteinizing hormone. Biol. Reprod. 4:129.

Knight, J. W., F. W. Bazer, and H. D. Wallace. 1973. Hormonal regulation of procine uterine protein secretion. J. Anim. Sci. 36:546.

Knight, J. W., F. W. Bazer, and H. D. Wallace. 1974. Effect of progesterone induced increase in uterine secretory activity on development of the porcine conceptus. J. Anim. Sci. 39:743.

Krishnan, R. S. and J. C. Daniel, Jr. 1967. "Blastokikin:" inducer and regulator of blastocyst development in the rabbit uterus. Science. 158:490.

Krzymowski, T., J. Kotwica, S. Okrasa, T. Dobozynska, and A. Ziek. 1978. Luteal function in sows after unilateral infusion of PGF2α into the anterior uterine vein on different days of the oestrus cycle. J. Reprod. Fertil. 54:21.

Lasely, J. F. 1968. Estrous cycles. In: E. S. E. Hafez (Ed.), Reproduction in Farm Animals. Lea and Febiger, Philadelphia, PA.

Lauderdale, J. W. 1972. Effects of PGF2α on pregnancy and estrous cycle of cattle. J. Anim. Sci. 35:246 (Abstr.).

Lerner, E. H., D. T. Mayer, and J. F. Lasely. 1957. Early embryonic mortality in strain crossed gilts. Mo. Agr. Exp. Sta. Res. Bull. 629.

Liehr, R. A., G. B. Marion, and H. H. Olson. 1972. Effects of prostaglandin on cattle estrous cycles. J. Anim. Sci. 35:247 (Abstr.).

Lodge, G. A. and R. M. McPherson. 1961. Level of feeding during early life and subsequent reproductive performance of sows. Anim. Prod. 3:19.

Longnecker, D. E. and B. N. Day. 1972. Maintenance of corpora lutea and pregnancy in unilaterally pregnant gilts by intrauterine infusion of embryonic tissue. J. Reprod. Fertil. 31:171.

Macmillan, K. L. and H. D. Hafs. 1968. Pituitary and hypothalamic endocrine changes associated with reproductive development of Holstein bulls. J. Anim. Sci. 27:1614.

Masuda, H., L. L. Anderson, D. M. Henricks, and R. M. Melampy. 1967. Progesterone in ovarian venous plasma and corpora lutea of the pig. Endocrinol. 80:240.

Matsuo, H., Y. Baba, R. M. G. Nair, A. Arimura, and A. V. Schally. 1971. Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. Biochem. Biophys. Res. Comm. 43:1334.

Matsuo, H., A. Arimura, R. M. G. Nair, and A. V. Schally. 1971. Synthesis of the porcine LH- and FSH-releasing hormone by the solid-phase method. Biochem. Biophys. Res. Comm. 45:822.

Mavrogensis, A. P. and O. W. Robison. 1976. Factors affecting puberty in swine. J. Anim. Sci. 42:1251.

Moor, R. M. and L. E. A. Rowson. 1966. The corpus luteum of the sheep: functional relationship between the embryo and the corpus luteum. J. Endocrinol. 34:233.

Murray, F. A., Jr., F. W. Bazer, H. D. Wallace, and A. C. Warnick. 1971. Cyclic nature of porcine uterine protein secretions. J. Anim. Sci. 32:388 (Abstr.).

Murray, F. A. and A. P. Grifo, Jr. 1976. Development of capacity to secrete progesterone-induced protein by the porcine uterus. Biol. Reprod. 15:620.

Neville, W. J., P. F. Scanlon, and J. W. McPherson. 1971. Maintenance of pregnancy in prepubertal gilts. J. Anim. Sci. 33:262 (Abstr.).

Niimura, S. and K. Ishida. 1978. Histochemical demonstration of prostaglandin dehydrogenases in the uterine and chorionic epithelia of the pregnant pig. J. Reprod. Fertil. 54:149.

Niswender, G. D., P. J. Dziuk, C. C. Kaltenbach, and H. W. Norton. 1970. Local effects of embryos in the uterus on corpora lutea in gilts. J. Anim. Sci. 30:225.

Niswender, G. D., L. E. Reichert, Jr., and D. R. Zimmerman. 1970. Radioimmunoassay of serum levels of luteinizing hormone throughout the estrous cycle in pigs. Endocrinol. 87:576.

Parlow, A. G., L. L. Anderson, and R. M. Melampy. 1964. Pituitary follicle-stimulating hormone and luteinizing hormone concentrations in relation to reproductive stages of the pig. Endocrinol. 75:365.

Polge, C., L. E. A. Rowson, and M. C. Chang. 1966. The effect of reducing the number of embryos during stages of gestation on maintenance of pregnancy in the pig. J. Reprod. Fertil. 12:395.

Puglisi, J. A., G. B. Rampacek, and R. R. Kraeling. 1978. Corpus luteum function following subtotal hysterectomy in the prepubertal gilt. J. Anim. Sci. 46:707.

Ramirez, V. D. and C. H. Sawyer. 1966. Changes in hypothalamic releasing factor (LRF). Endocrinol. 79:432.

Ramirez, V. D. 1973. Endocrinology of puberty. In: R. O. Greep and E. P. Astwood (Eds.), Handbook of Physiology Sect. 7 Endocrinology Vol. II. Am. Physiol. Soc., Washington, D.C.

Rampacek, G. B., R. R. Kraeling, and G. D. Ball. 1976. Luteal function in the hysterectomized prepubertal gilt. J. Anim. Sci. 43:792.

Rayford, P. L., H. J. Brinkley, and E. P. Young. 1971. Radioimmunoassay determination of LH concentration in the serum of female pigs. Endocrinol. 88:707.

Reddy, V. B. 1957. Some genetic aspects of fertility and an experimental modification of the intro-uterine environment. Ph.D. Dissertation, University of Missouri.

Reeves, J. J., A. Arimura, and A. V. Schally. 1970. Studies on dose response relationship of luteinizing hormone releasing hormone (LH-RH) in sheep. J. Anim. Sci. 31:933.

Reeves, J. J., A. Arimura, and A. V. Schally. 1971a. Pituitary responsiveness to purified luteinizing hormone-releasing hormone (LH-RH) at various stages of the estrous cycle in sheep. J. Anim. Sci. 32:123.

Reeves, J. J. A. Arimura, and A. V. Schally. 1971b. Changes in pituitary responsiveness to luteinizing hormone-releasing hormone (LH-RH) in anestrous ewes pretreated with estradiol benzoate. Biol. Reprod. 4:88.

Reiter, E. O., A. W. Root, and G. E. Duckett. 1976. The response of pituitary gonadotropes to a constant infusion of luteinizing hormone-releasing hormone (LH-RH) in normal prepubertal and pubertal children and in children with abnormalities of sexual development. J. Clin. Endocrinol. Metab. 43:400.

Reiter, E. O., A. W. Root, and G. E. Duckett. 1977. LH and FSH levels in urine and serum of prepubertal and pubertal children receiving a 3 hour infusion of LH-RH. J. Clin. Endocrinol. Metab. 44:56.

Rippel, R. H., R. E. Mauer, and W. F. White. 1972. Response of the prepubertal ewe to gonadotrophic releasing-hormone. J. Anim. Sci. 35:252 (Abstr.).

Rippel, R. H., E. S. Johnson, and W. F. White. 1974. Effect of consecutive injections of synthetic gonadotrophin releasing hormone on LH release in the anestrous and ovariectomized ewe. J. Anim. Sci. 39:907.

Robertson, G. L., R. H. Grummer, L. E. Casida, and A. B. Chapman. 1951. Age at puberty and related phenomena in outbred Chester-White and Poland-China gilts. J. Anim. Sci. 10:647.

Rowson, L. E. A. and R. M. Moor. 1967. The influence of embryonic tissue homogenate infused into the uterus, on the lifespan of the corpus luteum in sheep. J. Reprod. Fertil. 13:511.

Salisburg, R. K., R. F. Williams, E. C. Segerson, R. E. Fellows, and L. C. Ulberg. 1975. Function of CL in hysterectomized prepubertal gilts. J. Anim. Sci. 41:378 (Abstr.).

Sammelwitz, P. H., P. J. Dziuk, and A. V. Nalbandov. 1956. Effects of progesterone on embryonic mortality of rats and swine. J. Anim. Sci. 15:1211 (Abstr.).

Sammelwitz, P. H., J. P. Aldred, and A. V. Nalbandov. 1961. Mechanisms of maintenance of corpora lutea in pigs and rats. J. Reprod. Fert. 2:387.

Schlegel, W., L. M. Demers, H. E. Hildebrandt-Stark, H. R. Behrman, and R. O. Greep. 1974. Partial purification of human placental 15-hydroxyprostaglandin dehydrogenase: kinetic properties. Prostaglandins. 5:417.

Schomberg, D. W. 1967. A demonstration in vitro of luteolytic activity in pig uterine flushings. J. Endocrinol. 38:359.

Segal, D. H. and R. D. Baker. 1973. Maintenance of corpora lutea in prepubertal gilts. J. Anim. Sci. 37:762.

Segerson, E. D., Jr. 1976. Control of ovarian function with synthetic luteinizing hormone releasing factor (LRF) in the cyclic ewe, cyclic beef heifer and prepubertal gilt. Dissertation Abstracts Int. 36B:6003.

Self, H. L., R. H. Grummer, and L. E. Casida. 1955. The effects of various sequences of full and limited feeding on the reproductive phenomena in Chester-White and Poland-China gilt. J. Anim. Sci. 14:573.

Snedecor, G. W. and W. C. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa.

Sorensen, A. M., Jr., W. D. Thomas, and J. W. Gossett. 1961. A further study of the influence of level of energy intake and season on reproductive performance of gilts. J. Anim. Sci. 20:347.

Spies, H. G., D. R. Zimmerman, H. L. Self, and L. E. Casida. 1959. The effect of exogenous progesterone on formation and maintenance of the corpora lutea and on early embryo survival in pregnant swine. J. Anim. Sci. 18:163.

Spies, H. G., D. R. Zimmerman, H. L. Self, and L. E. Casida. 1960. Effect of exogenous progesterone on the corpora lutea of hysterectomized gilts. J. Anim. Sci. 19:101.

Spies, H. G., A. L. Slyter, and S. K. Quari. 1967. Regression of corpora lutea in pregnant gilts administered antiovine LH rabbit serum. J. Anim. Sci. 26:768.

Squiers, C. D., G. E. Dickerson, and D. T. Mayer. 1952. Influence of inbreeding, age and growth rate of sows on sexual maturity, rate of ovulation, fertilization and embryonic survival. Mo. Agr. Exp. Sta. Res. Bull. 494.

Squire, G. D., F. W. Bazer, and F. A. Murray, Jr. 1972. Electrophoretic patterns of porcine uterine protein secretions during the estrous cycle. Biol. Reprod. 7:321.

Stabenfeldt, G. H., E. L. Akins, L. L. Ewing, and M. C. Morrisette. 1969. Peripheral plasma progesterone levels in pigs during the estrous cycle. J. Reprod. Fertil. 20:443.

Staigmiller, R. B., N. L. First, and L. E. Casida. 1974. Compensatory growth and function of luteal tissue following unilateral ovariectomy during early pregnancy in pigs. J. Anim. Sci. 39:752.

Symons, A. M., N. F. Cunningham, and N. Saba. 1974. The gonadotrophic hormone response of anoestrous and cyclic ewes to synthetic luteinizing hormone-releasing hormone. J. Reprod. Fertil. 31:11.

Tanabe, T. Y., A. C. Warnick, L. E. Casida, and R. H. Grummer. 1949. The effects of gonadotrophins administered to sows and gilts during different stages of the estrual cycle. J. Anim. Sci. 8:550.

TerHaar, M. B. 1978. Luteinizing hormone responsiveness to luteinizing hormone releasing hormone in the adult guinea pig: direct ovarian involvement. J. Endocrinol. 76:49.

Thibault, C., M. Courot, L. Martinet, D. Mauleon, F. duMesnil du Buisson, R. Ortavandt, J. Pelletier, and J. P. Signoret. 1966. Regulation of breeding season and estrous cycles by light and external stimuli in some mammals. J. Anim. Sci. 25 (Suppl.):119.

Tillson, S. A. and R. E. Erb. 1967. Progesterone concentration in peripheral blood plasma of the domestic sow prior to and during early pregnancy. J. Anim. Sci. 26:1366.

Vandenbergh, J. G. 1969. Male odour accelerates female sexual maturation in mice. Endocrinol. 84:658.

Warnick, A. C., E. L. Wiggins, L. E. Casida, R. H. Grummer, and A. B. Chapman. 1951. Variation in puberty phenomena in inbred gilts. J. Anim. Sci. 10:479.

Wetteman, R. P., D. M. Hallford, D. L. Kreider, and E. J. Turman. 1977. Influence of prostaglandin F2α on endocrine changes at parturition in gilts. J. Anim. Sci. 44:106.

Wilson, L., Jr., R. J. Kenedella, R. L. Butcher, and E. K. Inskeep. 1972. Levels of prostaglandin in the uterine endometrium during the ovine estrous cycle. J. Anim. Sci. 34:93.

Wiltbank, J. N. and L. E. Casida. 1956. Alteration of ovarian activity by hysterectomy. J. Anim. Sci. 15:134.

Wright, P. J., G. Jenkin, R. B. Heap, and D. E. Walters. 1978. Pituitary responsiveness to LH-RH and TRH and the effects of progesterone or progesterone and oestradiol treatment in anoestrous sheep. J. Reprod. Fertil. 52:343.

Zimmerman, D. R., R. Carlson, and L. Nippert. 1969. Age at puberty in gilts as effected by daily heat checks with a boar. J. Anim. Sci. 29:203 (Abstr.).

VITA

Thomas Wayne Dowling was born on September 15, 1952 in Staten Island, New York. He attended elementary school and began high school there and graduated from high school in Scotch Plains, New Jersey. He received the Bachelor of Science degree from Rutgers University in 1974.

After undergraduate school, he was employed with Bio/Dynamics Inc. of East Millstone, New Jersey. In June 1975, he married the former Sharon Lee Kansky. He returned to Rutgers as a part-time graduate student the following Fall.

In the Fall of 1976, he transferred to Virginia Polytechnic Institute to continue his graduate program and transferred again in January 1978 to The University of Tennessee, Knoxville to complete his Master of Science degree in Animal Science.

He is now employed as a laboratory technician with the College of Veterinary Medicine at The University of Tennessee until his future is better determined.