STUDIES ON THE
Timing and the Mechanism
of Boar Induced
First Estrus in the
Pre-Pubertal Gilt

A thesis presented to the
Faculty of Graduate Studies and Research
in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy
in the Department of
Veterinary Physiological Sciences
University of Saskatchewan
Saskatoon, Saskatchewan

by

Dianna Lyn Kingsbury
September, 1992

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STUDIES ON THE TIMING AND THE MECHANISM OF BOAR INDUCED FIRST ESTRUS IN THE PRE-PUBERTAL GILT

The object of this study was to improve our understanding of the process of boar induced first estrus in gilts.

A technique for catheterization of the vena cava was refined and validated using valium and ketamine as anaesthetics.

In a study to determine if hormone levels in pre-pubertal gilts could be used to predict age at first estrus, gilts were blood sampled every three weeks, between 60 and 144 days of age, exposed to mature boars at 135 days of age, and monitored for estrus. Gilts studied during summer and responding to boar exposure with estrus before 200 days of age showed elevated LH concentrations at 81 days of age compared to gilts which failed to exhibit first estrus. FSH concentrations decreased in all gilts over time.

A second study was conducted to determine which aspects of the pulsatile release of LH from the pituitary were increased at 81 days of age in gilts responding to boar exposure with first estrus. However, gilts which showed or failed to show first estrus had similar LH pulse amplitude, LH pulse frequency and basal serum LH concentrations.

To study which aspect of the gilt’s reproductive hormone profile is affected by boar exposure, serum concentrations of LH, FSH, estradiol and cortisol were measured in control, responding and non-responding gilts following introduction of a boar. In
gilts responding to boar exposure with estrus, a transient increase in LH pulse frequency, mean LH and basal LH concentrations occurred immediately following boar introduction. FSH was lower in boar exposed gilts compared to controls gilts 10 and 20 days after boar introduction. Serum concentrations of cortisol were highest at all time periods in non-responding gilts. Estradiol concentrations were not affected by boar exposure.

Hormone therapy has been investigated as a possible solution to induce first estrus in gilts. Exogenous LH was given 4 times daily to gilts from 75 to 85 days of age. Basal serum LH concentrations were increased in treated gilts but there was no difference in age at first estrus between the control and treated gilts.
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ABSTRACT

The object of this study was to improve our understanding of the process of boar induced first estrus in gilts. A technique for catheterization of the vena cava was refined and validated using valium and ketamine as anaesthetics. In a study to determine if hormone levels in pre-pubertal gilts could be used to predict age at first estrus, 70 gilts were blood sampled every three weeks, between 60 and 144 days of age, exposed to mature boars at 135 days of age, and monitored for estrus. Gilts studied during summer and responding to boar exposure with estrus before 200 days of age showed elevated LH concentrations at 81 days of age compared to gilts which failed to exhibit first estrus. FSH concentrations decreased in all gilts over time. A second study was conducted to determine which aspects of the pulsatile release of LH from the pituitary were increased at 81 days of age in gilts responding to boar exposure with first estrus. However, gilts which showed or failed to show first estrus had similar LH pulse amplitude, LH pulse frequency and basal serum LH concentrations. To study which aspect of the gilt's reproductive hormone profile is affected by boar exposure, serum concentrations of LH, FSH, estradiol and cortisol were measured in control, responding and non-responding gilts following introduction of a boar. In gilts responding to boar exposure with estrus, a transient increase in LH pulse frequency, mean LH and basal LH concentrations occurred immediately following boar introduction. FSH was
lower in boar exposed gilts compared to controls gilts 10 and 20 days after boar introduction. Serum concentrations of cortisol were higher at all time periods in non-responding gilts. Estradiol concentrations were not affected by boar exposure. Hormone therapy was investigated as a possible solution to induce first estrus in gilts. Exogenous LH was given 4 times daily to gilts from 75 to 85 days of age. Basal serum LH concentrations were increased in treated gilts but there was no difference in age at first estrus between the control and treated gilts.
SUMMARY

The object of this study was to improve our understanding of reproduction in the pig and to determine what areas of replacement gilt management could be altered to improve efficiency. To this end, several studies were conducted to determine whether the timing of first estrus could be predicted in young pre-pubertal gilts, to determine what aspect of the pre-pubertal gilt's hormone profile was altered in response to boar exposure and to determine whether administration of exogenous Luteinizing Hormone (LH) could increase the percentage of gilts exhibiting first estrus.

A non-surgical technique for the catheterization of gilts was refined and validated using valium and ketamine as anaesthetics. The anaesthetics depressed LH pulse amplitude but this effect disappeared within 48 hours.

In a study to determine if hormone levels in pre-pubertal gilts could be used to predict age at first estrus, gilts between the ages of 60 and 144 days were blood sampled every three weeks at 60, 81, 102, 123 and 144 days of age. At 135 days of age, gilts began continuous exposure to mature boars and were checked daily for external signs of estrus until 200 days of age. During summer, but not winter, gilts which responded to boar exposure by exhibiting first estrus before 200 days of age showed elevated (P<0.008) mean serum luteinizing hormone (LH) concentrations at 81 days of age compared to boar exposed gilts which failed to exhibit first estrus.
Either one blood sample taken at day 81, or four blood samples, one each at morning and evening of days 81 and 82, yielded data that were suggestive of an ability to predict which gilts would exhibit estrus before 200 days of age.

Mean serum Follicle Stimulating Hormone (FSH) concentrations decreased in all gilts over time but no consistent relationship with age at first estrus was found. Gilts exposed to a boar which failed to respond with first estrus had lower mean serum FSH concentrations, until 102 days of age, than boar exposed gilts which did respond to boar exposure with estrus. Mean serum estradiol concentrations were similar in all groups of gilts until 144 days of age. At 144 days of age, gilts which responded to boar exposure with early estrus had higher (P < 0.0001) estradiol concentrations than gilts which showed estrus later or not at all.

These hormone data suggest that, with additional research regarding seasonal effects, LH concentrations could be used to identify, and mark for culling, pre-pubertal gilts which will fail to exhibit first estrus before 200 days of age. This would result in increased gilt management efficiency.

A second study was conducted to determine which aspects of the pulsatile release of LH from the pituitary were increased at 81 days of age in gilts responding to boar exposure with first estrus. Gilts were catheterized and blood samples were collected every 15 minutes for 8 hours at 62, 81 and 104 days of age. While some age related differences were
found in mean serum LH concentrations, both boar exposed gilts which showed or failed to show first estrus had similar LH pulse amplitude, LH pulse frequency and basal serum LH concentrations. It is possible that the stress of multiple catheterizations between 60 and 105 days of age interfered with the ability of the hypothalamic-pituitary-gonadal axis to generate any increase in serum LH concentrations. This conclusion is supported since 50% of gilts in this study failed to exhibit first estrus compared with 25% of gilts in the remainder of the studies.

Boar exposure is an integral part of swine management since it is capable of inducing first estrus in gilts 20 to 40 days earlier than in the absence of introduction of a male. The aspect of the gilt’s reproductive hormone profile affected by boar exposure is not known. To study this, serum concentrations of LH, FSH, estradiol and cortisol were measured in control gilts not exposed to a boar, gilts exposed to a boar at 134 days of age and which responded by displaying their first oestrus at 169 ± 5 days of age and in gilts which failed to respond to the boar and did not show estrus by 200 days of age.

Boar introduction produced a transient increase in LH pulse frequency, lasting no longer than 20 days (P < 0.001). Basal and mean serum LH concentrations were also increased (P < 0.05) in the boar-exposed gilts that displayed early estrus, but only in the six hours after boar introduction. Mean serum concentrations of FSH were lower in boar-
exposed gilts than in control gilts when measured 10 and 20 days after boar introduction (P < 0.05). Serum concentrations of cortisol decreased over the day of boar introduction in all groups of gilts (P < 0.05) and were highest at all time periods in gilts exposed to a boar but not showing oestrus by 200 days of age (P < 0.05). Serum concentrations of estradiol were not affected by boar exposure but were quite variable in gilts exposed to a boar but not cycling by 200 days of age.

These data suggest that the presence of an intact male stimulates an increase in LH pulse frequency over a 10 day period in prepubertal gilts, and this increase may stimulate some gilts to begin cycling before 200 days of age. The results also suggest that the high cortisol levels measured in the gilts exposed to boars but not cycling by 200 days of age may have interfered with the gilt's ability to respond to the boar with a sufficient increase in LH pulse frequency, thus delaying the onset of puberty. An increase in cortisol secretion would not appear to mediate the boar induced oestrus in gilts.

Hormone therapy was investigated as a possible solution to induce first estrus in gilts. Based on the results that suggested that serum concentrations of LH at 81 days may well be predictive of the time of first estrus, exogenous LH was given 4 times daily to gilts from 75 to 85 days of age. It was hypothesized that increasing mean serum LH concentrations in all gilts around 80 days of age would increase the percentage of gilts
exhibiting first estrus. Basal serum LH concentrations were increased two-fold at 81 days of age but there was no difference in age at first estrus between the LH-treated gilts (148.9 ± 1.9 days) and control gilts (154.2 ± 3.4 days).

The percentage of gilts exhibiting estrus in the control group (N=7) was 60% while 80% of gilts in the LH treatment group (N=8) exhibited estrus. However, this difference was not significant due to the small number of gilts in each group. This study requires repetition with increased numbers of animals in each group and investigation of the impact of higher doses of exogenous LH.
DEDICATION

This work is dedicated to SPENSER, who was not present for the beginning, who gave me everything in the middle, who could not fight on to celebrate the end.

I miss you.

B.I.S.S. Ch. Namatta’s Ichiban of Skeldale, C.D.X., W.C.
June 5, 1989 - June 22, 1992

This work is also dedicated to HAWKEYE, who was taken from me on what should have been a day of celebration. May your next guardian angel be more vigilant; may your heaven be filled with bald porcupines.

I’m sorry that I failed you.

Namatta’s Steady Teddy, C.D.X., W.C.
December 18, 1989 - October 3, 1992
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LIST OF ABBREVIATIONS

ACU  Animal care unit
ASP250  Auriomysin sulfamethazine penicillin
CV  Coefficient of variation
E<sub>c</sub>  Estradiol-17β
EB  Estradiol benzoate
EOP  Endogenous opioid peptide
FSH  Follicle stimulating hormone
GnRH  Gonadotropin releasing hormone
hCG  Human chorionic gonadotropin
HND  High nutrient density
IHU  Initial housing unit
IM  Intramuscular
IU  International unit
IV  Intravenous
L  Landrace
LH  Luteinizing hormone
MH  Malignant hyperthermia
NIH  National Institute of Health (USA)
PMSG  Pregnant mare serum gonadotropin
POMC  Propiomelanocortin
PRL  Prolactin
PSRC  Prairie Swine Research Centre
PULSAR  Pulse analysis program
SAS  Statistical Analysis System
T  Time
WCVM  Western College of Veterinary Medicine
Y  Yorkshire
1.0. Introduction

Within a given year approximately 25% of the sows in commercial swine operations are culled due to decreasing productivity. This decline is primarily associated with an increase in feed intake as the animal ages, an increase in the number of still-born piglets, and an overall higher incidence of reproductive failure compared to younger sows. Ovarian cysts, which can arise spontaneously during the estrus cycle and after parturition, also contribute to reproductive failure in sows (Hall et al., 1989).

To maintain economic efficiency, a producer will replace sows, with gilts, after four to six litters. Gilts are ideally managed to farrow a large litter before they reach one year of age; to accomplish this, females must exhibit their first estrus before 200 days of age, since mating is usually allowed at the second or third estrus and gestation length is approximately 114 days. Productive management also relies on good maintenance of body condition throughout the first lactation so the post-weaning to estrus interval is short. Thus, the economic viability of swine operations depends on management of gilts such that a sufficient number are available when needed.

Current management techniques involve the intensive raising of feeder pigs in confinement, until they reach market age and weight at approximately 155 days and 105 kilograms (1991 figures; Saskatchewan
Pork Producers Board). They are then slaughtered and sold for consumption. Gilts selected to become part of the replacement herd are kept longer, since, in the absence of males, they do not exhibit their first behavioral estrus until between 200-230 days of age (Kirkwood and Hughes, 1979; Kirkwood and Hughes, 1981). These extra 45 days are very costly in terms of food and housing. Further expense is incurred since 25% of selected replacement gilts will either exhibit such irregular estrous cycles that breeding is not recommended or else will fail to show external signs of estrus before 200 days of age. Thus even more gilts than will be needed as replacements must be kept past market weight to determine their reproductive capabilities. Therefore, a replacement gilt herd will number 35% of the total number of sows to ensure an adequate supply of healthy cycling females.

Stockyard penalties for overweight gilts that are not used as sow replacements amount to approximately ten dollars for every five kilograms over market weight (100-105 kg; 1991; B. Andries, PSRC, Saskatoon, Saskatchewan). This can amount to about $30 per gilt or about $2400 per year in a 250 sow herd. Reduction in the number of extra gilts retained past market weight requires an alteration in management procedures or a reliable method of testing for reproductive capability at an early age.
2.0. Review of the literature

2.1. Introduction

Much research has centred on refining gilt management to ensure that a high percentage of animals exhibit first estrus early and to reduce the number of non-productive animals. Practically oriented studies have focused on the effects of housing, nutrition and boar stimulation on the onset of estrous cycles, while basic studies have focused on hormonal stimulation of first estrus and hormone patterns associated with sexual development and puberty. However, few studies have been conducted to determine if reproductive hormone patterns are indicative of eventual reproductive status.

2.2. Sexual development in the gilt

2.2.1. Definitions

Puberty is the stage of sexual development when an animal is capable of naturally participating in a fertile mating. As such, puberty is a very finite phase of sexual maturation. The latter encompasses a lengthy time frame and involves not only reproductive tract development but also the development of the cognitive centres of the brain associated with reproductive behaviour. Sexual development extends beyond puberty with
the final growth of reproductive organs to adult size, the increase in secretion of hormones to adult levels and the addition of sexual experience.

Estrus is the period of sexual receptivity exhibited by a female animal. The duration of estrus varies between domestic species and is designed to coordinate sperm deposition and capacitation with ovulation. The mean interval for standing estrus in a mature cycling sow is 50 hours with a range of 8 to 136 hours (Huang et al., 1992). The inter-estrus interval is the length of time between two subsequent periods of sexual receptivity. In gilts, the inter-estrus interval is 21 ± 3 days.

Delayed puberty is a condition when gilts have exceeded 240 days of age before exhibiting normal estrus (Edqvist et al., 1978). Additionally, serum progesterone concentrations remain below 250 pg/ml. Often, delayed puberty can be overcome through the administration of exogenous hormones, indicating that the reproductive tract is fully developed, physically, and is waiting for an appropriate endocrine cue to begin functioning (Edqvist et al., 1978; Lutz et al., 1985).

Many mammals often experience precocious ovulation, ovulation which occurs without a concomitant expression of estrus. Conversely, estrus can occur with ovulation but without appropriate behaviour; these are termed "silent estrus" periods. Both of these events are often associated with the pre and peri-pubertal periods and neither can result in a fertile mating.
2.2.2. Sexual development in gilts

2.2.2.1. Hormone patterns from birth to puberty in gilts

Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) secretion patterns change dramatically throughout development of the gilt (see Figure 2.1) and are correlated with growth and development of the reproductive tract. At 80 days of gestation FSH can be detected in the fetal pituitary (McNamara et al., 1985) and primary follicles are recognizable on the fetal ovary (Oxender et al., 1979). The fetal hypothalamus stimulates the pituitary (Bruhn et al., 1983) to begin secreting pulses of LH by day 81 of gestation (Ponzilius et al., 1986) doubling the basal serum concentration (Bruhn et al., 1983). By day 100 of gestation serum LH concentrations begin to drop and continue to decline until 10 weeks post-partum (Elsaesser et al., 1976).

At birth serum FSH levels and LH pulsatility decline rapidly (Pelletier et al., 1981) about the same time that secondary follicles first appear in the ovary (Oxender et al., 1979). After birth, serum FSH values start to increase culminating in a peak at 54 days of age (Camous et al., 1985). Serum LH concentrations drop immediately after birth, stay low until 60 days of age and then rise gradually to a maximum concentration sustained between 75 and 130 days of age (Camous et al., 1985).
**Figure 2.1:** Schematic patterns of plasma prolactin, LH, FSH, and urinary estrone in gilts from birth to puberty. (From Camous *et al.*, 1985).

LH and FSH pulses appear again at 8-10 weeks and rise to a maximum frequency between 82-125 days of age (Pelletier *et al.*, 1981). Tertiary follicles emerge between 62 and 90 days of age (Oxender *et al.*, 1979), followed later by a decline in LH levels, presumably as a consequence of ovarian development and the establishment of estradiol (E$_2$) mediated negative feedback.

As the gilt approaches puberty, LH pulse frequency declines (Diekman and Trout, 1984) and there is a transient drop in serum FSH concentrations (Diekman *et al.*, 1983). Estradiol levels increase as the gilt
approaches puberty (Diekman and Trout, 1984) and, just before the onset of estrus, FSH concentrations drop as estradiol values peak (Ponzilius et al., 1983). During this period, however, there is no change in the number of estradiol or progesterone receptors in either the pituitary or hypothalamus (Diekman and Anderson, 1982). This suggests that estradiol positive feedback may not be mediated through a change in receptor numbers.

Immediately before estrus the frequency of low amplitude LH pulses rises (Lutz et al., 1984). The resultant LH surge is accompanied by an FSH peak on day 0 of estrus and a second FSH peak on day 3 (Ponzilius et al., 1984). A preovulatory increase in progesterone concentration occurs but is not essential for estrus (Diekman and Trout, 1984).

A four stage increase (see Figure 2.2) in urinary estrone occurs as the gilt matures, beginning at 40 days of age and ending with display of first estrus at 230 days (Camous et al., 1985). It is postulated that either the relative metabolism of estradiol to estrone increases as the gilt matures or else the synthesis of estradiol increases over time resulting in an increase in metabolite excretion. The latter is consistent with the hypothesis that, if estradiol concentrations increase during development, then gilts which fail to exhibit estrus before 200 days of age will present a different $E_2$ profile compared to gilts which do exhibit estrus before 200 days of age.
Figure 2.2: Pattern of urinary estrone excretion per 24 hours in one representative gilt. Lines across the graph indicate the four developmental stages of estrone secretion. (From Camous et al., 1985).
Despite the large amount of information regarding the hormonal changes associated with puberty, little is known about the hormonal profiles of gilts which fail to exhibit first estrus before 200 days of age as compared to profiles from gilts which do show estrus by 200 days of age. The most dynamic changes in hormone patterns in gilts which show estrus are seen between birth and 125 days of age. The peak in serum LH and FSH pulse frequencies at approximately 90 days of age, coupled with the emergence of tertiary follicles at the same time, indicates that this could be a crucial stage in sexual maturation. It may also be a step which is missed or is atypical in gilts which fail to exhibit estrus or which cycle irregularly. Similarly, if the sequential rise in estrone is paralleled by increasing serum estradiol concentrations, then this too could possibly reflect a fundamental mechanism of puberty. If so, it may be possible to predict adult reproductive status based on prepubertal hormone profiles.

In addition to the commercial implications of a blood test to predict first estrus in the gilt, information regarding correlations between the the timing of first estrus and serum hormone concentrations would be useful in determining the key physiological mechanism involved in the normal onset of puberty and could lead to treatment for animals which have failed to begin regular estrous cycles. Hormone therapy and efficacious boar exposure could ensure that virtually all gilts in the replacement herd would
be potential replacement stock, thereby eliminating the cost of maintaining surplus animals.

2.2.2.2. Reproductive tract development in gilts

The growth pattern of the reproductive tract from birth to puberty is divided into four stages: 0-70 days of age, 70-140 days of age, 140 days until puberty and then the changes associated with puberty (Dyck and Swierstra, 1983). Within each stage, the ovaries, oviducts, uterus, and pituitary, as well as the thyroid and adrenal glands, grow and change at different rates. Before 70 days of age, growth of the ovaries and uterus is linear and associated with increased overall weight gain and age (Oxender et al., 1979; Dyck and Swierstra, 1983). The ovaries are populated primarily with primordial and primary follicles (Colenbrander et al., 1977; Oxender et al., 1979). A typical 46 day old gilt has 84% primordial follicles, 5% primary follicles, 2% secondary follicles and no tertiary follicles on each ovary (Oxender et al., 1979). The pituitary, thyroid and adrenal glands also increase in size as the gilt matures, but when weights are expressed as relative to body growth, the thyroid gland ceases to increase by 42 days of age and the weight of the adrenal gland relative to total body weight declines steadily after 70 days of age (Dyck and Swierstra, 1983).

Between 70 and 90 days of age, secondary follicles increase on the ovaries until they represent 30% of the total follicle number (Oxender et al.,
Increases in total ovarian weight were associated with this increase in the number of follicles (Dyck and Swierstra, 1983). Overall ovarian and uterine weights show a marked increase relative to total body weight by 84 days of age, a decrease relative to total body weight until 112 days of age and then remain stable until the peri-pubertal stage (Dyck and Swierstra, 1983). Associated with the increase in ovarian weight at 84 days is the appearance of the first tertiary follicles, sometime between 60 and 90 days of age (Oxender et al., 1979).

The time between 140 days and puberty is variable, and can be influenced by factors such as housing (Rampacek et al., 1984), nutrition and season (Christenson, 1986). There are no further visual changes in the ovaries, oviducts or uterine length, although uterine weight increases (Dyck and Swierstra, 1983). The relative percentages of primary, secondary and tertiary follicles remain similar to that seen at 90 days of age (Oxender et al., 1979). The entire reproductive tract appears quiescent, but mature, after 140 days of age, since stimulation with exogenous gonadotropins after 140 days of age results in ovulation and estrus (Baker et al., 1973; Guthrie, 1977; Oxender et al., 1979). However, artificial induction of estrus in gilts produces many unwanted side effects such as abnormal cyclicity and abnormal sexual behaviour (Paterson et al., 1984).

At puberty, oviduct and uterine weights increase markedly relative to total body weight probably due to the increased plasma estrogen levels.
associated with estrus (Dyck and Swierstra, 1983). Ovarian weight does not change at puberty since the greatest increase in the follicle supply has already occurred prior to puberty (Dyck and Swierstra, 1983). Weights of the thyroid, pituitary and adrenal glands all remain constant in the peri-pubertal phase (Dyck and Swierstra, 1983).

Of the four stages of reproductive tract growth in gilts (Dyck and Swierstra, 1983), the third, between 140 days of age and puberty is the most variable. The reproductive tract is physically mature and ready to begin regular estrous cycles at any time following 140 days but it is often a further 20 to 40 days before puberty. This time may be necessary for the completion of negative steroid feedback mechanisms and hypothalamic maturation, both of which are hypothesized to be stimulated by transport stress and boar exposure.

2.2.2.3. Development of sexual behaviour in gilts and boars

Dimorphic sexual behaviour in pigs is evident at 1 month of age and is at its greatest at 2 months of age (Berry and Signoret, 1984). A period follows where young males will not only mount females in estrus but will allow mature males to mount them as well (Signoret et al., 1989). Young gilts exhibit far less sexual activity than do males of the same age (Berry and Signoret, 1984) and show little variation in their sexual behaviour at puberty. Males, however, undergo a two stage process of defeminization, or
loss of inclination to display female-typical behaviours, at or just before puberty (Ford, 1990). Proceptivity, the solicitation of mature males, is defeminized in young males before receptivity to being mounted by mature males (Ford, 1983; Adkins-Regan et al., 1989). Both events occur as a result of the increase in testicular steroids associated with puberty (Ford, 1990).

In other mammals, the brain remains undifferentiated with respect to sexual behaviour only until late gestation and early life (Ford, 1990). Masculinity in males is then produced by testicular secretions of androgens which travel to the brain and are aromatized into estrogen (Ford, 1990). The combination of androgens and estrogen in the male brain during late gestation produce sexual behaviour differentiation (Ford, 1990). Differentiation of female sexual behaviour, however, is likely due to constant low levels of estrogens in early life (Ford, 1990).

2.2.3. Puberty in gilts

2.2.3.1. The gonadotrophin pulse generator

The onset of puberty is characterized by an increased frequency of low amplitude serum LH pulses (Lutz et al., 1984). As in monkeys, sheep, rats and most other mammals (Karsch, 1987) this pattern of pulsatile LH release in pigs is presumed to be determined by the pulsatile release of gonadotrophin releasing hormone (GnRH) from the hypothalamus (Kraeling
and Barb, 1990). GnRH pulses, induced experimentally in ewes (Clarke et al., 1984; Legan et al., 1985) by pulsatile injection, produce marked changes in LH pulse amplitude and frequency and led early researchers to conclude that it was pulsatile GnRH which was responsible for the unique pulsatile patterns of luteinizing hormone secretion (Karsch, 1987). It is known that each LH pulse is always accompanied by a pulse of GnRH but the converse, that all GnRH pulses are accompanied by LH surges, is not true (Karsch, 1987).

The source of the pulsatile release of GnRH is thought to be electrical automaticity of neurons in the medial basal hypothalamus, since electrical activity in this area of the brain in sheep and monkeys corresponds with LH release from the pituitary (Thiery and Pelletier, 1981; Wilson et al., 1984). Additionally, alterations of LH pulsatile release in the monkey results in changes in electrical activity in the medial basal hypothalamus (Kaufman et al., 1985) and, in the pig, anaesthetization of the central nervous system with pentobarbitone sodium blocks or delays the pre-ovulatory LH surge (Parvizi et al., 1976). However, unlike the magnocellular oxytocin neurons of the posterior pituitary (Lincoln and Wakerley, 1974; Dreifuss et al., 1981) which are known to exhibit rhythmic electrical activity, no individual cells which secrete GnRH in the hypothalamus have been shown to possess rhythmic electrical activity (Karsch, 1987).
Study of GnRH neurons is difficult as the cells are very small. Nevertheless, it is presumed that there exist populations of GnRH producing cells in the hypothalamus which rhythmically secrete pulses of GnRH which in turn stimulate the anterior pituitary to secrete LH in a pulsatile fashion (Karsch, 1987).

Connections between these GnRH producing cells have not yet been established (Karsch, 1987). Two theories for connection and coordination of electrical activity are promoted. The first suggests that the GnRH cells are regulated by pacemaker neurons (Dreifuss et al., 1981) while the second suggests that each GnRH cell possesses its own electrical activity and that groups of GnRH producing cells are linked together for communication (Karsch, 1987). Difficulties with both theories exist as GnRH producing cells are scattered diffusely throughout a large area of the hypothalamus (Witken et al., 1982) in patterns which are species specific (Karsch, 1987). The role of the diverse sub-populations of GnRH cells is not known. Some may be involved in sexual behaviour (Kendrick and Dixson, 1985) while others may be responsible for the stimulation of pituitary hormone release (Karsch, 1987).

2.2.3.2. Establishment of negative feedback of gonadal steroids

In 1965, Ramirez and McCann proposed a mechanism for the maturational changes in the hypothalamic-pituitary-gonadal axis associated
with puberty. The "gonadostat hypothesis" states that there is a threshold for negative feedback in the hypothalamus called the gonadostat. This gonadostat increases as the animal matures resulting in a decreased sensitivity to the negative effects of gonadal steroids. With less negative feedback, the serum concentrations of LH and FSH increase as the animal ages.

Removal of the ovaries from young gilts at one week of age does not result in an increase in serum concentrations of LH, likely because tertiary follicles, which produce estrogen, are not yet present on the ovary (Oxender et al., 1979). The decrease in serum LH concentrations that occurs between 7 and 35 days of age occurs in ovariectomized and intact gilts, suggesting that ovarian steroids are not involved at this age (Elsaesser et al., 1978). At 8 weeks of age, the hypothalamic-pituitary axis of the male pig is responsive to testicular steroids (Colenbrander et al., 1977) and it is extrapolated that the female system is responsive to ovarian steroids at the same age (Elsaesser, 1982). Ovariectomy anytime after 60 days results in increased serum concentrations of LH and FSH suggesting that removal of the ovaries at this age removes the negative feedback influence of follicular estrogens (Elsaesser, 1982).

The amount of estrogen required, and the duration of exposure required to depress serum concentrations of LH decreases as the gilt matures (Elsaesser, 1982). In 6 day old gilts 600 µg of estradiol benzoate
per kg of body weight was needed to suppress mean serum LH
concentrations (Elsaesser and Parvizi, 1979), whereas in 53 to 70 day old
gilts, 60 µg of estradiol benzoate produced the same effect (Pomerantz et al.,
1975; Foxcroft et al., 1975).

The specific site of estradiol feedback on the pituitary and
hypothalamus is not known. Investigations which focused on the isolation
of estradiol receptors in these two areas of the brain showed no difference in
the number of receptors in a 30 day old gilt or a 168 day old gilt (Diekman
and Anderson, 1979). However, the capacity of the receptors for estradiol
does increase markedly in mature cycling gilts when compared to pre-
pubertal gilts (Diekman and Anderson, 1979).

2.2.3.3 Establishment of steroid positive feedback

The capacity to switch from negative steroid feedback to positive
steroid feedback is essential for the expression of puberty since positive
steroid feedback stimulates the preovulatory surge of LH and FSH from the
pituitary (Howland et al., 1978). Until just before puberty, steroid feedback
remains negative (Clark, 1987). Then, as the ovaries mature and follicle
production increases, the mean amount of estradiol in the serum increases.
When the hypothalamic-pituitary-gonadal axis is physically mature,
increased estradiol can feedback either positively (Karsch et al., 1987) or
negatively (Karsch et al., 1973) to the hypothalamus and pituitary
depending on the stage of the estrous cycle. At puberty, or at the peri-ovulatory stage in a mature gilt, the normally present estradiol negative feedback is transiently switched to a positive signal (Karsch, 1987), resulting in an increase in serum concentrations of LH and FSH. LH stimulates the theca cells on the developing follicles to produce androgens which then travel to the granulosa cells of the follicle. In the granulosa cells, the androgens are converted to estrogens. This increase in the concentration of estradiol from the developing follicle is accompanied by an increase in the number of FSH receptors on the granulosa cells. The combination of high estradiol levels and large numbers of FSH receptors results in the production of LH receptors on the granulosa cells. The developing follicle is now primed for and is able to be sensitive to an LH surge. This cycle occurs until the follicle develops into a mature Graafian follicle and ovulates. Following ovulation, steroid feedback is once again negative.

Several maturational steps are involved in the establishment of positive steroid feedback. The first step occurs around 60 days of age, when tertiary follicles first appear (Oxender et al., 1979). At this age, administration of estradiol benzoate to a gilt will positively or negatively affect LH secretion. However, in 160 day old gilts, estradiol benzoate produces consistent positive affects on LH secretion (Elsaesser and Foxcroft, 1978). Another step which occurs around 60 days of age, involves the
reduction in the amount of time needed for the pituitary to respond to estradiol by increasing the secretion of LH (Elsaesser and Foxcroft, 1978).

A third step in the establishment of positive steroid feedback is the decrease in the number of pre-ovulatory surges of LH as the gilt matures into a productive sow. In immature gilts, two and even three pre-ovulatory surges of LH occur over a 12 hour period (Elsaesser, 1982) mostly during times of darkness. Mature cyclic sows are also known to have nocturnal surges of LH (Parvizi et al., 1976) but most sows will show only one pre-ovulatory surge, especially after they have produced, lactated and weaned their first litter (Elsaesser and Parvizi, 1980).

2.2.3.4. Endogenous opioid peptides and reproduction

Endogenous opioid peptides (EOPs) are compounds which naturally occur in the brain (Weiner et al., 1988). The first to be discovered was a porcine enkephalin which produced morphine-like responses in intestinal bioassays (Hughes et al., 1975). Following this discovery, three main groups of opioids have been described. The first group, endorphins, are derived from the C terminal portion (see Figure 2.3) of the pituitary product, proopiomelanocortin (POMC) (Nakanishi et al., 1979). Also derived from POMC are α- and β-melanocyte stimulating hormone and β-lipoprotein (see Figure 2.3). The most common endorphin is β-endorphin, a 31 amino acid peptide, located in high concentrations in the pituitary (Bloom et al., 1977).
β-endorphins are also found in the hypophyseal portal system in much greater concentrations than in the peripheral plasma, leading to the proposition that β-endorphin may be a neurohormone (Wardlaw et al., 1980).

Figure 2.3: Proopiomelanocortin and its various peptide products. (From Hughes et al., 1975).

The second group of EOPs are 5-amino acid peptides called enkephalins. The two major peptides are methionine-enkephalin and leucine-enkephalin (Hughes et al., 1975). Enkephalins are found in various areas of the mammalian body including the hypothalamus, the limbic system, the spinal cord and the gastrointestinal tract (Brooks et al., 1986).
and are postulated to have a neurotransmitter function (Osborne et al., 1978; Brooks et al., 1986).

Dynorphins are 32 amino acid peptides first isolated from porcine pituitaries (Goldstein et al., 1979; Fischli et al., 1982). Dynorphin A and dynorphin B are found in high concentrations in the posterior pituitary and the hypothalamus and in smaller concentrations in the spinal cord and the gastrointestinal tract (Brooks et al., 1986). Many neurons which contain enkephalins also cross-react with antisera against dynorphins indicating that dynorphins may also have a neurotransmitter role (Brooks et al., 1986).

EOPs suppress LH secretion in several species including sheep (Malven, 1986), cattle (Whisnant et al., 1986), and rats (Bhanot and Wilkinson, 1983), by tonically inhibiting the effects of estradiol feedback in the pituitary (Armstrong et al., 1988; Kraeling and Barb, 1990) and inhibiting the release of GnRH (Brooks et al., 1986). In the sexually mature gilt, administration of the opioid antagonist, naloxone, results in increased LH secretion during the luteal, but not the follicular, phase of the estrous cycle (Barb et al., 1986). However, in mature ovariectomized gilts, LH secretion is unaffected by naloxone unless progesterone is also given (Barb et al., 1988). Since the luteal phase is dominated by progesterone secretion, it has been suggested that EOPs require a progesterone dominated milieu to produce their effects on LH secretion (Estienne et al., 1989; Kraeling and Barb, 1990).
When intact and ovariectomized, progesterone treated, prepubertal gilts were treated with naloxone, there was no effect on LH or FSH secretion (Barb et al., 1988). However, in gilts ovariectomized prepubertally, and then treated with progesterone and naloxone at the chronological age when contemporary gilts entered puberty, LH secretion increased (Barb et al., 1988). Barb concluded that EOP inhibition of LH secretion in the prepubertal gilt is a maturational process of the brain and that, while progesterone may be required for activation, the entire process is independent of the ovaries (Kraeling and Barb, 1990).

The neurons which manufacture EOPs in the brain are postulated to be independent of the GnRH neurons which dictate the pulsatile release pattern of LH (Kraeling and Barb, 1990). Alternatively, as in rats, the EOP neurons may be linked to the GnRH neurons, but activation of the system may be delayed until the gilt attains puberty (Kraeling and Barb, 1990).

Thus, the maturation of the steroid negative and positive feedback systems in the prepubertal gilt appears to involve ovarian independent maturation of part of the brain. The influx of progesterone from the first corpus luteum may be part of the stimulus for the final activation of the EOP system. The requirement for progesterone activation of EOP maturation may also explain why the first few cycles of the young gilt are somewhat irregular in the timing, duration and magnitude of the hormonal events. By the third estrous cycle, sufficient progesterone has been
produced to mediate the EOP maturation in the brain and the hormonal
events of the estrous cycle are those of a mature gilt.

2.2.3.5. Summary
The development of the negative and positive estrogen feedback
systems are crucial events for the expression of normal puberty. Feedback
on LH likely occurs at the level of the hypothalamus and specifically
involves EOP modulation of the GnRH pulse generator (Kraeling and Barb,
1990) in the mature gilt. In the prepubertal gilt, maturation of EOP
modulation of LH secretion is likely dependent upon the presence of
progesterone for final maturation, but is generally independent of the
ovaries. FSH is likely not affected by endogenous opioid peptides as its
secretion is under the negative effects of follicular inhibin (Kraeling and

2.3. Hormonal acceleration of first estrus in the gilt

2.3.1. Administration of exogenous hormones
Administration of exogenous hormones was theoretically expected to
be an expedient method of inducing synchronous estrus in pre-pubertal
gilts. Hormones such as gonadotropin releasing hormone (GnRH), pregnant
mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG), and others, have all been tried with various degrees of success. However, problems with side effects and inconsistent results make these techniques of questionable practical value.

Boar exposed gilts exhibiting signs of delayed puberty have been successfully treated with an intravenous injection of 1 mg GnRH; 3 of 4 exhibited estrus within 7 days of treatment and, on necropsy, presented normal ovaries (Edqvist et al., 1978). In all four gilts, serum LH concentrations rose steadily to a peak between 60 and 90 minutes post-injection and progesterone levels increased normally. The fourth gilt failed to ovulate, had lower LH values, and displayed a general lack of follicular growth and no luteal tissue. However, in a concurrent study (Guthrie, 1977), GnRH treatment (I.M., 500 µg) failed to induce estrus in 150-160 day old gilts. This dichotomy of results could be attributed to the different breeds used in the two studies, the different means of hormone administration or the different ages of the gilts. The results also suggest that the focus of GnRH therapy should be on treating delayed puberty and not on stimulating early estrus.

Administration of GnRH to simulate the LH pulse frequency, amplitude and mean serum concentrations seen at the normal onset of puberty, can stimulate precocious ovulation, but not normal puberty, in 75 kg gilts (Lutz et al., 1985). Only one of the experimental animals showed a
normal estrous cycle and subsequently reovulated. The authors concluded that, while gilts are capable of being mature at 164 days of age, hypothalamic maturation is incomplete, and thus the gilt is not able to begin normal estrus cyclicity.

PMSG and hCG have also been used, individually and in combination, to synchronize estrus in gilts. The combination of the two hormones can produce high ovulation rates and estrus (Baker et al., 1973; Guthrie, 1977) as well as conception and farrowing 6-8 weeks earlier than average (Schilling and Cerne, 1972). However, synchrony of estrous was maintained for only 2 cycles (Schilling and Cerne, 1972).

hCG (250 I.U.) alone produced a decrease in the interval between transport stress and the onset of estrus in 190 day old gilts, but did not alter the percentage of gilts exhibiting first estrus (Esbenshade and Day, 1981). PMSG (1000 I.U.) alone has stimulated estrus and ovulation, in 190 day old gilts weighing 100 kg, within 80 hours of treatment (Dial et al., 1984). Increases in the dose beyond 10 I.U./kg served to further increase the number of gilts with appropriate endocrine responses during the pre- and peri-ovulatory periods (Dial et al., 1984).

While administration of estradiol benzoate (EB) can stimulate LH surges (Dial et al., 1984), especially in 160 day old gilts (Fleming and Dailey, 1985), the surges are not often associated with ovulation (Kirkwood and Aherne, 1988). Estradiol benzoate in gilts which do subsequently
ovulate and exhibit normal estrus does produce higher embryonic survival rates than does the combination of PMSG and hCG (Paterson et al., 1984).

Despite the promising nature of these data and the possibilities of obtaining early synchronous estrus in young prepubertal gilts, it has been determined that while treating with PMSG and hCG may stimulate ovulation in a high percentage of gilts, not all of those gilts exhibit normal cyclicity and very few show normal behavioural estrus (Paterson et al., 1984). Similar side effects have been seen with administration of estradiol benzoate (Yang et al., 1987; Kirkwood and Aherne, 1988). Other contraindications, such as estrus without ovulation and cystic follicles have also been reported (Dyck, 1988). Thus, while treatment with exogenous hormones is simple and cost effective initially, there are difficulties over the long term. Stimulating gilts of poor reproductive potential to ovulate and farrow has little commercial value, since delayed puberty is a heritable genetic trait (Edqvist et al., 1978) and any resultant offspring are not likely to enter the replacement gilt herd.

2.3.2. Immunization against reproductive hormones

With advances in the area of antibody research it is now possible to immunize domestic species against various hormones involved in hormonal feedback and regulation of reproductive events. Both early and efficient stimulation and cessation of reproductive events can be achieved. However,
some undesirable side effects and inconsistencies require further investigation.

Androstenedione is produced in the thecal cells of new follicles and aromatized to estradiol in the granulosa cells. Immunological removal of androstenedione is postulated to change steroid feedback in the hypothalamic-pituitary-ovarian axis such that gonadotropin levels are increased, follicular development and ovulation rate are enhanced, and puberty stimulated (Sivesindtajet et al., 1989). Conversely, androgens appear to be involved in the process of follicular atresia. By removing their influence it is postulated that the rate of atresia is decreased and thus follicles are stimulated to ovulate (Sivesindtajet et al., 1989). Whatever the mechanism, immunization against androstenedione has proven successful in gilts (McKinnie et al., 1988) and ewes (Scaramuzzi et al., 1980; Gibb et al., 1981; Sivesindtajet et al., 1989). Ovulation rates as high as 24% above average with no effect on pregnancy maintenance or embryo survival have been reported (McKinnie et al., 1988). However, in some gilts, immunization against androstenedione has produced no effects on onset of ovulation, puberty or ovulation rate (Walton, 1985) and, in cattle, the same treatment produced nymphomania and ovarian cysts. In both gilts and heifers low variable titres, coupled with factors as diverse as genetic and individual variation, body condition and environment, make immunization
against androstenedione of questionable benefit for stimulating early estrus (Reeves et al., 1989).

While active immunization of heifers, ewes and goats against estradiol can produce a marked increase in ovulation rate (Reeves et al., 1989), a high percentage of the females treated display cystic follicles and become acyclic (Rawlings et al., 1978; Wise and Schanbacher, 1983).

More promising with respect to increasing female fecundity is immunization against inhibin. Inhibin, produced in the developing follicle, feeds back at the level of the pituitary gland to decrease FSH secretion. Removal of its influence eliminates this feedback, and theoretically circulating FSH increases. Extra FSH stimulates follicular development and ovulation rate (Reeves et al., 1989). Ewes immunized against partially purified inhibin from bovine follicular fluid ovulated at a rate twice that of control animals (Henderson et al., 1984; Cummins et al., 1986). Further purification of the inhibin enabled researchers to immunize ewes against a 30 amino acid fragment of the molecule with the result being an ovulation rate 3-4 times above normal (Forage et al., 1987; Schanbacher, 1988). The exact mechanism by which this increase in ovulation rate is achieved is unknown, since immunization against inhibin does not appear to produce an increase in serum FSH concentrations (Reeves et al., 1989) but it is possible that an increase was not detected due to the sampling regimes used in the studies. Further investigation on the long term effects is also necessary.
An increase in serum concentration of FSH can be produced in ewes by passive immunization with antibodies against LH (Fitzgerald et al., 1985). Along with this increased FSH, cycle length was increased as was ovulation rate. This, along with data from studies investigating active immunization against LH, suggests that the increase in ovulation rate is due to an increase in circulating FSH concentrations (Roberts and Reeves, 1988; Reeves et al., 1989). However, high antibody titres against LH blocks ovulation in both ewes and heifers (DeSilva et al., 1986; Johnson et al., 1988) and boosters can prolong the contraceptive nature of the anti-LH for up to one year (Grieger and Reeves, 1988). Similar contraceptive results are produced in bulls, but not rams or boars, immunized against LH. Bulls show reduced testes size and regressed secondary sex characteristics but the actual existence or period of sterility has not been investigated (Reeves et al., 1989).

2.3.3. Long term implications of hormone therapy

Although many of the drug treatment possibilities seemed initially successful in stimulating first estrus and ovulation in the gilt, side effects such as abnormal behaviour and cyclicity make hormone therapy both undesirable and economically inefficient for the commercial acceleration of first estrus in gilts (Yang et al., 1987; Dyck, 1988; Kirkwood and Aherne, 1988). In addition, there is growing dissatisfaction among consumers
regarding administration of drugs to food animals and possible chemical residues. This has caused a resurgence of research interest in the mechanism of the traditional method of stimulating first estrus in the gilt, boar exposure.

2.4. General determinants of age at first estrus in the gilt

2.4.1. Seasonality and photoperiod

Empirical observations and scientific study have determined that swine are polyestrous polytocos mammals. However, there is circannual variation in both the achievement of physical maturity and estrous cycle regularity. Research has shown that gilts assessed in the summer months have less reproductive tract development than do age matched gilts assessed in the winter (Christenson, 1986). Gilts maturing in the summer also exhibit their first estrus, naturally or in response to boar stimulation, at a later age (Marvogenis and Robinson, 1976; Christenson, 1981; Hughes et al., 1990) than gilts maturing in the fall or winter. Studies indicate, however, that seasonal variations in reproductive capacity appear to be overcome by olfactory stimulation from boars since boar exposed gilts show close to 100% estrus in any season, while gilts not exposed to boars show a marked decrease in the percentage entering estrus in the summer months (Booth and Baldwin, 1983; Paterson et al., 1989; Hughes et al., 1990).
Darkness can cause (Ntunde et al., 1979) or not cause (Awotwi and Anderson, 1985) a delay in puberty in the gilt. However, it has also been reported that a photoperiod with lengthened light hours does not advance puberty or alter the ovulation rate (Ntunde et al., 1979; Diekman and Hoagland, 1983; Awotwi and Anderson, 1985), has no effect on LH, FSH or prolactin (PRL) concentrations, but does hasten estrus in fall maturing gilts (Diekman and Hoagland, 1983). In climates with extended hours of darkness then, it would be advantageous to ensure adequate lighting is available to prevent any possible delays in sexual maturity (Ntunde et al., 1979).

Cyclic sows can experience a period of anestrus in the summer and some mature males have lower sperm counts and lower libido when the temperature is high (Claus and Weiler, 1985). Landrace gilts, cross-bred and reciprocal bred gilts generally exhibit their first estrus earlier than do other breeds of pigs (Allrich et al., 1985), suggesting that boar induced age at first estrus may be breed specific.

In lactating sows, a long photoperiod of 16L/8D, does not alter serum concentrations of prolactin, LH or growth hormone (Kraeling et al., 1983). Basal serum LH and prolactin concentrations are also unaffected by long photoperiods in ovariectomized gilts.
2.4.2. Housing

Management can have a profound effect on the timing of first estrus in the gilt. Pigs are generally raised in confinement, a system that has been shown to delay puberty (Christenson, 1981; Rampacek et al., 1981; Rampacek et al., 1984). Non-confined animals have higher basal concentrations of luteinizing hormone and respond to boar exposure within a shorter time frame than do confined gilts (Rampacek et al., 1984; Caton et al., 1986).

Gilts also require a minimum number of other females with which to interact in order for a high percentage of gilts to exhibit estrus by 9 months of age (Christenson, 1984). Groups of 9, 18 or 27 per pen resulted in 78 - 81% of gilts showing estrus, while groups of 3 gilts per pen only resulted in 57% of gilts exhibiting estrus (Christenson, 1984). However, when group size was held constant and the amount of individual space allotted was decreased, there was no detrimental effect on the percentage of gilts showing estrus (Ford and Teague, 1978).

Cleanliness can also have a marked impact on age at first estrus. A study completed by Malayer and colleagues (1987) determined that manure gases, especially ammonia, at levels four times normal reduced the percentage of gilts in estrus by 200 days of age. The LH and FSH secretion profiles of both the control and gas-exposed gilts were similar. Malayer et al., (1987) concluded that the ammonia probably interfered with the gilts
ability to perceive and react to the pheromonal cues from the boars to which they were exposed. Desensitization of the olfactory system, then, seems to have as severe a consequence on puberty as does removal of the olfactory bulbs themselves (Kirkwood et al., 1981).

2.4.3. Nutrition

Adequate supplies of nutrients, especially protein, calcium and phosphorus, are essential for normal growth and development. Studies have shown that restricting food intake in growing gilts, and thus decreasing calcium and phosphorus intake below that required for maximum bone strength, delays puberty (Patience and Thacker, 1989). In addition, decreasing nutrient intake leads to a decrease in backfat reserves; this too may result in abnormal sexual maturation.

Gilt age appears to be the most accurate indicator of the degree of sexual development (Hughes and Cole, 1976) but one report indicates that gilts need to reach a critical body weight, not age, before puberty can be stimulated (King, 1989). A study utilizing constant chronological age compared with constant weight showed that gilts respond with increased variability to external stimuli when the independent variable is weight (Hughes and Cole, 1976). In addition, excess body fat tends to cause a delay in estrus in gilts (King, 1989) as it does in women (Frisch, 1984; see also below).
The common industry practice of "flushing gilts", restrict feeding until about 10 days before estrus and then increasing the amount of foodstuffs available (Aherne et al., 1976), appears to have no effect on age at first estrus and is of no use when the goal is to increase litter size (Kirkwood et al., 1988).

Ewes, ovariectomized to remove the effects of negative steroid feedback and inhibit, have pituitary glands which are more responsive to GnRH stimulation as long as food intake was moderate (Rhind et al., 1989). However, GnRH secretion and hypothalamic activity are not affected by the level of food intake (Rhind et al., 1989). Diets deficient in linoleic and linolenic acids can delay puberty in rats due to a reduced availability of arachidonic acid for metabolite synthesis, the result of which is retarded development of the hypothalamus and ovaries (Smith et al., 1989).

Results of studies involving human females report that primary or secondary amenorrhoea results from extreme physical training associated with an inadequate caloric intake or excessive dieting (Frisch, 1984). Return to normal cyclicity is only achieved once the fat to lean ratio in the body is increased (Frisch and McArthur, 1974; Frisch, 1980). Excess fat in humans (Hartz et al., 1979) and cattle (Marshall and Peel, 1908) can also interrupt sexual cyclicity, leading to the conclusion that "normal" body fat levels are essential for normal reproduction.
The combination of a short day photoperiod (8L/16D) and restricted nutrition can result in significant reductions in the size of the reproductive organs in male deer mice, while body mass and total body water content generally remain unaffected (Nelson et al., 1992). Short days and low food availability, such as would occur during the winter months, explains the genesis of the decreased reproductive capability of the mouse during the winter.

2.5. Effect of exposure to males on estrus in females

2.5.1. The mouse, the ram, and the bull

Estrus can be efficiently stimulated in ewes by introducing mature intact rams at appropriate times (Riches and Watson, 1954; Watson and Radford, 1960). This "ram effect" was mediated through a marked increase in LH release from the pituitary (Martin et al., 1978; Poindron et al., 1980) and the effect itself can be mimicked by exposing the ewes to male fleece or wool extracts (Knight and Lynch, 1980). Vasectomized rams have also been successfully used to stimulate ewes to make the transition from anaestrous to estrus (Hudgens et al., 1987), with the result being an increase in the number of ewes ovulating, earlier mating and lambing.

Similar effects of males on female reproduction have been described in other species as well. Beef heifers exposed to male urine exhibit puberty
earlier than control animals exposed to water (Izard and Vandenburg, 1982). Female grey short-tail opossums rarely show estrus unless they are exposed to intact or, to a lesser degree castrated, males (Fadem, 1987). Female mice are equally reactive to males of their species as are prairie deer mice, collard lemmings and meadow voles (Marchlewksa-Moj, 1984). In each of these species, the males are capable of stimulating a return to estrus in a previously anestrus female or of stimulating early puberty.

2.5.2. The "Boar Effect"

2.5.2.1. Patterns of exposure

The simplest, and most effective, method of stimulating early estrus in the gilt is boar exposure. Studies focusing on the effectiveness of fence-line contact between gilts and boars compared to full physical contact, determined that daily, full physical contact results in stimulation of first estrus in 40% more gilts than does fence-line contact (Deligeorgis et al., 1984; Caton et al., 1986). Further studies suggested that, like in mice (Drickamer, 1987), total time of contact between boar and gilt was less important than the frequency of contact (Hughes et al., 1990). Frequencies of less than 3 days per week did not adversely affect the percentage of gilts exhibiting estrus but did result in a marked increase in the number of days
between initial boar exposure and the exhibition of first estrus (Paterson et al., 1989).

The minimum length of daily exposure required between a gilt and a boar, to stimulate first estrus in the gilt, was long thought to be 30 minutes (Hughes, 1982; Van Lunen and Aherne, 1987). However, more recent work has determined that a daily minimum exposure of 5 to 10 minutes was sufficient to stimulate first estrus in the gilt (Caton et al., 1986; Paterson et al., 1989) and that 10 minutes daily will result in the shortest interval between initial exposure and first estrus (Caton et al., 1986; Paterson et al., 1989).

Patterns of boar exposure usually require, at some point, movement of the gilts either to new larger pens or to the boar pen. The gilt’s response to the boar is better if exposure occurs in the male’s pen than in the gilts’ pen (Scheimann et al., 1976; Van Lunen and Aherne, 1987) most likely due to a build-up of pheromones in the boar pen (Kirkwood and Hughes, 1980a). However, gilts moved to a boar pen which was cleaned daily did not show a reduced percentage of estrus compared to gilts moved to a regular boar pen (Pearce and Hughes, 1985). This suggests that either pheromone build-up is not the reason for the better results in the boar’s pen or that the cleaning regime employed was insufficient to remove all traces of pheromones from the previous day.
2.5.2.2. Optimal age of exposure in the gilt

Gilts are generally reared with male and female littermates until weaning at 28 days of age. Following separation from the sow, gilts are generally isolated from males until the optimum age of between 135 and 170 days of age (Hughes and Cole, 1976; Kirkwood and Hughes, 1979).

Raising gilts from birth through maturity in the presence of intact or castrated males can be inconsequential in terms of age at first estrus (Paterson and Lindsay, 1980; Eastham et al., 1984) or can delay puberty (Cole et al., 1982). This delay in puberty is possibly due to the gilts being too immature to respond to boar stimulation before 130 days of age or that the gilts become habituated to the stimulus of the male and therefore are unable to respond at a later age (Kirkwood and Hughes, 1982). However, habituation has been discounted, since gilts exposed continuously to a boar respond to novel boar exposure in the same way as gilts never before exposed to a male (Eastham and Cole, 1982).

Seventy eight percent of gilts raised in fence-line contact with a mature male until 160 days of age prior to relocation and exposure to a novel male exhibited first estrus compared to 39% of non-relocated gilts managed in the same way (Eastham and Cole, 1987). This indicates that relocation is essential for the boar effect to work if a mature boar is not novel (Hughes et al., 1990).
2.5.2.3. Boar Age

The age of the boar to be used for gilt stimulation is an important component of the "boar effect". Only mature males of at least 11 months of age are capable of producing the effect (Kirkwood and Hughes, 1980; Kirkwood and Hughes, 1981). It has been postulated that males of less than 6 months of age are unable to synthesize or release the C19 pheromonal steroids implicated in the olfactory portion of boar stimulation (Kirkwood and Hughes, 1981). However, young boars have stimulated first estrus in gilts, given sufficient time (probably for the male to mature). Ovulation rate at first estrus in gilts exposed to young males was no different than that seen following stimulation by a mature male (Kirkwood and Hughes, 1981), suggesting that no detrimental effects on fertility were produced by exposure to immature boars.

Differences exist in serum and saliva concentrations of pheromones between males since pheromones increase with age (Booth, 1975), group rearing (Narendran et al., 1980) and previous sexual experience (Hughes et al., 1990). Between breed differences also exist in males as evidenced by the Göttingen miniature boar, which has concentrations of salivary pheromones 10 to 20 times higher than those seen in domestic boars (Booth, 1984). But, despite these between boar variations, there is little known about the difference in each boar's ability to stimulate first estrus in a gilt once he attains maturity (Hughes et al., 1990).
2.5.3. Mechanism of male stimulation of estrus in the female

2.5.3.1. The general mechanism

The mechanism by which males stimulate females to begin normal estrous cycles is thought to be pheromonal in nature, since often, exposure of the female to urine will produce the same effect as the physical presence of the male (Marchlewska-Moj, 1984). Pheromones are secretion products of skin glands or they are found in the urine or faeces of males. They are substances which, when secreted by one animal, evoke a specific reaction in another animal of the same species (Karlson and Luscher, 1959). Mammalian pheromones are generally species specific and produce a well characterized endocrinological or behaviourial reaction (Beauchamp et al., 1976).

There are two broad classes of pheromones to which domestic species respond. Signal pheromones result in an immediate change in motor activity while priming pheromones stimulate both hormonal and neurohormonal activity (Marchlewska-Moj, 1984).

Males of different species produce different pheromones. In male mice, testosterone is required for the production of pheromones secreted in the urine (Marchlewska-Moj, 1984). Also, production of pheromones diminishes when males are castrated, but, through testosterone treatment, production can be restored (Marchlewska-Mohj, 1984). This suggests that
the chemicals are either androgenic in nature or that their production is androgen dependent (Fadem, 1987; Marchlewksa-Moj, 1984).

The synthesis site of pheromones is unknown. Urine, without accessory gland secretions, can stimulate females (Drickamer and Murphy, 1978), suggesting that the kidney may be involved in their production. However, sites as diverse as the liver and the preputial glands have been implicated in pheromone production (Marchlewksa-Moj, 1984).

Pheromones interact with either the olfactory epithelium in the nasal cavity or the secondary sensory vomernasal organ in the nasal passages of most domestic species (Marchlewksa-Moj, 1984). Both sensory systems have separate nervous connections to the brain and some have direct connections into the limbic system.

Animals may react to the detection of some pheromones with a characteristic flehman (Wysocki, 1979) or a pumping response (quick inhalation and exhalation), which serves to increase the flow of the air or liquid borne chemicals across or through the olfactory sensors. Following detection, the signal from the pheromones is thought to be transferred to the hypothalamus where it stimulates a release of GnRH and subsequently, LH in sheep (Poindron et al., 1980), and LH and prolactin in mice (Bronson and Desjardins, 1974; Bronson and Maruniak, 1976; Keverne, 1982). Chronic elevation of prolactin levels in juvenile female mice significantly advances puberty (Advis et al., 1981) by enhancing estrogen levels
sufficiently to stimulate the first ovulation. The rise in estradiol concentrations is postulated to trigger positive feedback to the pituitary and a pre-ovulatory surge of gonadotrophins (Hughes et al., 1990). Female grey short-tail opossums which responded to the presence of a male by exhibiting estrus had higher estrogen concentrations than did exposed females which failed to exhibit estrus (Fadem, 1989). This suggests that the hypothalamic-pituitary stimulation of ovarian estrogen production seen in marsupials in response to male pheromones is intricately involved in the normal resumption of estrus.

The presence of pheromones from an alien male is sufficient to interfere with implantation in the newly mated mouse and cause her to return to estrus for re-breeding (Dominic, 1966). This effect is mediated through failure of the corpus lutea. Decreased progesterone at this stage of pregnancy renders implantation impossible.

2.5.3.2. Mechanism of the "boar effect"

The mechanism of the boar effect is still a mystery. What is known is that removal of the olfactory bulbs of prepubertal gilts renders the animals insensitive to boar stimulation (Kirkwood et al., 1981) suggesting that a major part of the female’s receptivity system is olfactory in nature. The stress of moving gilts to novel surroundings is synergistic with the effect of boar exposure on age at first estrus (van Lunen and Aherne, 1987)
but stress itself is ineffective at inducing puberty (Pearce and Hughes, 1985). Pearce and Hughes (1987) reported that stress, generated by full physical contact with a mature boar, was reflected by a substantial rise in plasma cortisol levels, an increase which was postulated to be a necessary component of boar stimulated first estrus. But, more recently, Pearce and Paterson (1992) concluded that cortisol is not a mediator of boar induced estrus in the pre-pubertal gilt. Exposure of gilts to 3α-androstenol, a steroid in boar saliva, induced early estrus (Kirkwood and Hughes, 1983) but the steroid effect could not be separated from the effects of transport stress, so no causal relationship could be inferred.

Separation of the visual, auditory and tactile components of a boar, to determine the effect of each on the gilt, is difficult to achieve. Castrated or long-term androgenized castrates have been used in studies focussed on the visual appearance of the boar. However, in castrates, tissue distribution is altered compared to an intact male and the lack of testes may be important (Hughes et al., 1990). Androgenized castrate males are also likely to produce similar vocal sounds and body odour as would an intact male, again defeating the purpose of providing a visual picture without the other corresponding boar cues (Hughes et al., 1990). Two studies have focused on the visual aspect of the boar effect. The first determined that castrate males are as effective as intact males at stimulating first estrus in the gilt and concluded that the visual component of the boar effect is paramount.
over the olfactory (Kinsey et al., 1976). A more recent study, however, showed a marked decrease in the percentage of gilts responding with first estrus to a castrated boar compared to an intact boar (Pearce and Hughes, 1987b).

The Kinsey et al., (1976) report and the Pearce and Hughes (1987) report also disagree on the importance of auditory cues from the boar. Kinsey recorded boar chants, played them to pre-pubertal gilts and concluded that there was no difference in the number of gilts responding than if the gilts had been exposed to an intact male. Pearce and Hughes (1987) categorized the auditory stimulation component of the boar effect as minor at best; in fact boar chants had no effect, in combination with an androgenized castrate, on the percentage of gilts exhibiting first estrus (Pearce and Hughes, 1987).

Physical contact between boar and gilt is described as the best method for boar induction of first estrus (Deligeorgis et al., 1984; Caton et al., 1986). It is impossible to isolate tactile cues from a live boar, but, studies show that visual, pen-line, and facial contact is inferior to whole body contact in inducing first estrus (Pearce and Hughes, 1990). From these results, it is concluded that tactile stimulation plays a large role in boar induction of estrus in the gilt.

Studies involving the boar effect and the monitoring of reproductive hormone concentrations have not reported a rise in serum LH
concentrations in response to presence of the boar although some have reported increases in serum estradiol levels (Paterson et al., 1980; Esbenshade et al., 1982; Hughes et al., 1990). Hughes has proposed that the following mechanism may be responsible for the boar's effect on first estrus in the gilt:

1. Boar pheromones may alter the pattern of LH secretion.

2. The release of cortisol accompanying full boar contact further alters the secretory pattern of LH and/or physical contact with the boar enhances the transfer of primer pheromones.

3. These alterations in LH secretion stimulate follicular development and result in an elevation of plasma estradiol concentrations.

4. The estradiol stimulatory feedback system is triggered, resulting in the first preovulatory LH surge and thus the pubertal ovulation. (From Hughes et al., 1990.)

Thus, while several aspects of boar stimulation of first estrus in the gilt are under investigation, it is still not known what effect, if any, males have on the levels of reproductive hormones in the females. Theoretically, it is feasible to assume that exposure to boars results in either short term or long term alterations in the hormone profile of gilts which respond to the stimulation. It is also possible, then, to assume that gilts which fail to respond to boar stimulation do not present these same alterations. In any commercial management system, the usual practice of exposing gilts to mature boars to induce first estrus by 200 days of age is ineffective in a
small number of pigs. Why some animals should be unresponsive while others respond is not known. Examination of the hormone profiles of both responding and non-responding gilts may provide information regarding the mechanism of the "boar effect" and may also provide direction for possible hormone therapy of non-responding gilts.

2.6. Conclusions

Despite the available literature regarding the chemical induction of the first ovulation and boar stimulated first estrus in the gilt, there are still no statements regarding the timing or the nature of the hormonal signal involved in stimulation of first estrus or the reason for the failure of a large percentage of gilts to exhibit their first behavioral estrus before 200 days of age.

Several questions still remain regarding the induction of estrus in the gilt and the hormonal profiles associated with gilts which fail to exhibit external signs of estrus before 200 days. More information about the hormone levels in the non-cycling gilt could lead to the development of a predictive test to identify these gilts so they could be culled from the replacement gilt herd at market weight and age. This would result in significant savings for the commercial producer. Conversely, these non-cycling gilts could be converted to productive animals through hormone therapy. This, too, would result in economic benefit to the producer. From
a scientific stand-point, this information could help in identifying some of
the mechanisms involved in puberty.

2.7. Hypotheses

The following general hypotheses form the basis for this project.

1. That, in gilts less than 120 days old, the concentrations of LH, FSH, and
estradiol, individually or in combination, correlate with eventual age at first
estrus.

2. That changes in LH or FSH in gilts less than 120 days old reflect a
critical endocrine maturational change essential for the normal expression
of first estrus before 200 days of age.

3. That gilts which respond to boar exposure by exhibiting estrus before
200 days of age have a different hormone profile than do gilts which fail to
respond to exposure and that these differences represent the endocrine
mechanism of boar induced first estrus.
3.0. General Materials and Methods

3.1. Experimental Animals

All animals used in the course of this study were maintained in accordance with the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1980).

All gilts and boars were purchased from the Prairie Swine Research Centre (PSRC), University of Saskatchewan, Saskatoon, SK. The PSRC maintains a specific pathogen free (SPF) herd of Yorkshire (Y), Landrace (L) or Y x L, Y x (Y x L) or L x (Y x L) pigs and is managed to commercial productivity standards. All replacement gilts for the PSRC herd are evaluated and selected using the Beltsville Scoring System before entering the main herd.

3.1.1. Management of gilts housed at PSRC

From birth to weaning at 28 days, gilts were housed in farrowing pens (1.5m X 2.2m) with their dam and male litter mates. At two weeks of age, piglets were provided unlimited access to High Nutrient Density (HND) Start creep feed (Federated Co-operatives Limited, Saskatoon, SK). At three weeks, the ration was altered to half HND Start and half Pig Start (Federated Co-operatives Limited, Saskatoon, SK). At weaning, gilts were separated from male litter mates and group housed, 10 per pen (1.2m X
1.2m or 1.2m X 1.5m), in a weaner barn and fed, *ad libitum*, Pig Start. All feed was medicated with auriomycin sulfamethazine penicillin (ASP250) in a ratio of 110:110:50 respectively (Federated Co-operatives Limited, Saskatoon, SK).

At 55 days of age, gilts were moved from the weaner barn to a finishing barn and group housed 10 or 11 per pen (1.5m X 4.6m). Gilts had continuous access to feeders containing a barley-wheat-soybean finishing ration, supplemented with vitamins and minerals, formulated to provide at least 13 MJ DE/kg and 14% crude protein (Prairie Swine Research Centre, Saskatoon, SK). Water was provided continuously through nipple waterers. At 135 days of age, gilts were relocated to novel pens, within the same barn, adjacent to a pen housing a mature intact boar. Boar exposure was continuous until 200 days of age.

All PSC barns were windowless. Natural light only entered through slats in the ventilation fans. Banks of incandescent light bulbs were used to illuminate the barns for approximately one hour daily during feeding and inspection. In winter, the barns were heated to a maximum of 17-18°C. In summer, temperatures in the barn followed ambient temperatures.

3.1.2. Management of gilts and boars housed at the WCVM

All gilts were transported to the WCVM at least five days prior to being assigned to a specific study.
Gilts younger than 100 days of age were housed in B-Wing of the Animal Care Unit (ACU) of the Western College of Veterinary Medicine (WCVM). Groups of six gilts were confined to individual rooms (3.7m X 3.7m or 4.3m X 2.5m). Fluorescent lighting was set for 12 hours light and 12 hours darkness per day. Heat lamps were provided for all gilts less than 45 days old and water was provided continuously through nipple waterers. Gilts were generally provided with automatic feeders containing a grower ration (Pork-Gro, Federated Co-operatives Limited, Saskatoon, SK).

Gilts older than 100 days of age were housed in groups of 4-6 in pens measuring 4.6m X 2.5m. Water was supplied through nipple waterers. All gilts were fed Pork-Gro (Federated Co-operatives Limited, Saskatoon, SK).

Catheterized gilts were housed in calf-carts (0.75m X 1.5m) in the same room in which they had been previously housed or in individual pens (1.5m X 1.5m) adjacent to pens containing a mature boar. Each cart or pen was outfitted with a nipple waterer or a water dish. Food was supplied in small feeding dishes twice daily until blood collection was complete. Gilts remained in calf carts for a maximum of 54 hours.

At 135 days of age, all gilts to be exposed to boars were moved, either in calf-carts or herded on foot, to the Initial Housing Unit (IHU). In the IHU, pigs were housed either in groups of 4-6 in pens (2.5m X 3.1m, 12 hours L/12 hours D) adjacent to pens housing a mature boar of at least two years of age or in individual pens (1.5m X 1.5m) adjacent to pens containing
a mature boar of at least two years of age. All pen railings allowed full muzzle contact between boars and gilts.

Boars were housed in individual pens (2.5m X 3.1m) each outfitted with a nipple waterer and an automatic refill water bowl. Pork-Gro food was provided twice daily in large food bowls. In between experiments, boars were removed from their regular pens to equal sized pens with concrete floors to prevent any limb problems due to long term footing on tenderfoot flooring. Wounds, abscesses and pressure sores were treated topically with Topazine (Austin Laboratories (Canada Ltd., Joliette, Quebec). Boars were sedated and de-tusked once yearly in between experiments.

3.1.3. Marketing of gilts

All gilts were marketed to Intercontinental Packers (Saskatoon, SK) after exhibiting two regular estrous cycles or at 200 days of age. Gilts used in Chapter 4 were marketed at 210 days of age to comply with drug withdrawal time regulations. Boars were also marketed to Intercontinental Packers (Saskatoon, SK) at the conclusion of the research.

3.2. Collection and processing of blood samples

Blood was collected either by venipuncture of the vena cava (or nearby vein) or through catheters placed in the vena cava or an adjacent
vein. All samples were placed in clean borosilicate glass tubes (12mm X 75mm) and allowed to clot at room temperature for at least 4 hours. Tubes were then centrifuged (3000 g, 10 minutes), the clots removed and the serum re-centrifuged (3000 g, 10 minutes). All serum harvested was stored in plastic tubes at -20°C until analyzed.

3.3. Estrus Detection

Gilts were checked daily from 135 days of age to 200 days for external signs of estrus, which included a standing lordosis response and a red swollen vulva. First estrus was taken as the first of two consecutive periods of estrus $21 \pm 3$ days apart.

Gilts were subdivided into groups in two different ways depending on the objectives of the study. Gilts were divided into four groups according to the age at which they first exhibited first estrus: early group gilts exhibited first estrus between 140 and 160 days of age, middle group gilts exhibited estrus between 160-180 days of age, late gilts exhibited estrus between 180 and 200 days of age and non-cycling gilts failed to exhibit estrus before 200 days of age. Otherwise, gilts were grouped into two groups according to whether or not they responded or failed to respond to boar introduction by exhibiting first estrus before 200 days of age.
3.4. **Radioimmunoassays**

Serum concentrations of LH were determined in an ovine/bovine radioimmunoassay system (Rawlings et al., 1984; Currie and Rawlings, 1989) using the porcine standards USDA-pLH-B:1 AND USDA-pFSH-B-1, respectively.

Sensitivity of the LH assay, defined as the lowest concentration of standard different from zero ($P < 0.05$), was 0.06 ng LH ml$^{-1}$ serum, and the curve range was to 64 ng LH ml$^{-1}$ serum. Intra- (N=7) and inter- (N=21) assay coefficients of variation for LH were 9.2% and 10.7% or 9.8% and 15% respectively for reference sera replicated in each assay and with mean LH concentrations of 1.5 ng ml$^{-1}$ or 11.3 ng ml$^{-1}$ respectively. The high reference serum was made by adding 10 ng of porcine LH ml$^{-1}$ (USDA-pLH-B:1) to the low reference serum (measured difference $9.8 \pm 1.45$ ng ml$^{-1}$ (SD)).

Sensitivity of the FSH assay was 0.13 ng ml$^{-1}$, and the curve range was to 64 ng ml$^{-1}$ serum. Intra- and interassay coefficients of variation were 8.3% (N=7) and 8.3% (N=21) or 8.9% (N=7) and 8.7% (N=21) for reference sera replicated in each assay and with mean serum FSH concentrations of 8.5 ng ml$^{-1}$ or 13.5 ng ml$^{-1}$, respectively. The high reference serum was made by adding 5 ng of porcine FSH ml$^{-1}$ (USDA-pFSH-B-1) to the low reference serum (measured difference $5.0 \pm 0.45$ ng ml$^{-1}$ (SD)).

Serum estradiol concentrations were determined by established radioimmunoassay (Rawlings et al., 1984). Intra- and interassay coefficients
of variation were 18.5% (N=5) and 22.2% (N=10) or 2.2% (N=5) and 9.4% (N=10) for porcine reference serum with means of 5.4 pg ml⁻¹ or 16.3 pg ml⁻¹, respectively. The high reference sera was made by adding 10 pg of 17β-estradiol ml⁻¹ to the low reference serum (measured difference 10.9 ± 1.38 pg ml⁻¹, (SD)). Sensitivity of the assay was 1 pg ml⁻¹ (P < 0.05). The assay blank in the estradiol assay was not different from zero. Extraction efficiency was 86 ± 1.2% (SEM) for estradiol. Estradiol concentrations were determined for each gilt, in Chapter 7, from serum pools corresponding to each of the 4 sampling times: before and after introduction of the boar and then 10 and 20 days following continuous boar introduction.

Serum cortisol concentrations were determined directly, without extraction, using a fluorescence polarization immunoassay (TD₆ System, Abbott Laboratories, Irving, Texas). Crossreactions in this assay were 4.7% and 4.5% for 11 deoxycortisol and corticosterone respectively and < 0.1% for progesterone and testosterone. Sensitivity was 12.4 nmol⁻¹. The assay blank was not different from zero. Intra- and inter assay coefficients of variation were 8.42% and 7.23%, respectively, for a control serum (mean = 110.4 nmol⁻¹) replicated in every assay. When 50,100 or 200 nmol⁻¹ of cortisol were added to serum, concentrations of 57 ± 0.7, 109 ± 1.6 or 215 ± 3.3 nmol⁻¹ were estimated after subtraction of the concentration (106 ± 1.8 nmol⁻¹) of endogenous cortisol. Serum cortisol concentrations, in Chapter 7,
were determined for the 4h prior to introduction of the boar, for 1h immediately after introduction and then for a further 4h.

3.5. Statistical Analysis

Luteinizing hormone pulse amplitude, pulse frequency, mean and basal concentrations were determined using PULSAR (Pulse Analysis Program, PC-Pulsar, Version 2, Bethesda, Maryland; adapted for IBM-PC by James Gitzen and Victor Ramirez, University of Illinois, Urbana, Illinois). Mean values ± S.E. for any parameter were determined using the means procedure in the Statistical Analysis System (SAS, Version 6, S.A.S. Institute Inc., Cary, N.C.).

Homogeneity of variance for each set of data was determined using the Bartlett's test in SAS. When homogeneity was not present (P > 0.1) an appropriate transformation for the data, such that P < 0.1, was determined using the Box-Cox Procedure (BMDP Statistical Software, 1981, Berkeley, CA). Values were transformed before the results were analyzed. Data presented is raw and any annotation regarding significant differences was determined from analyzed transformed data.

The general linear models (GLM) analysis of variance procedure for repeated measures (SAS, Version 6) was used for most data and the T-test procedure for the remainder. Group effects were determined using orthogonal contrasts (Steel and Torrie, 1980; SAS, Version 6). Overall
group, time and age effects were determined with Tukey's and Scheffe's tests (SAS User's Guide: Statistics, 1985).

If several different breed crosses of gilts were used in a particular study, breed was incorporated into the model statement of the analysis of variance to determine its effect. At no time was breed of gilt a significant factor.
4.0. Development, validation and application of a non-surgical catheterization procedure.

4.1. Catheterization Procedure

4.1.1. Introduction

Restraint for the purposes of repetitive blood sampling over long periods of time is impractical and stressful in swine. Non-surgical sampling techniques such as multiple venipuncture of the vena cava require restraint and introduce a high degree of stress. In addition to being too small for repeated withdrawal of blood samples, access to both ear and tail veins is difficult for intensive sampling regimes in young gilts.

Several surgical procedures for cannulation of the jugular and femoral vein and the vena cava of anaesthetized pigs exist (Anderson and Elsley, 1969; Ford and Maurer, 1978; Karalus and Downey, 1989). However, the procedures are surgical, requiring sterile technique and often necessitate the use of halothane, an anaesthetic that is associated with malignant hyperthermia (Steffey, 1986). Additionally, surgical installation of catheters into the vena cava usually requires ligatures around the vein and sutures on the skin at the point of exteriorization (Christison and Curtin, 1969; Bailie et al., 1986). This system would preclude the simple removal of the catheters without further surgical intervention.
We required a rapid non-surgical catheterization procedure to enable the collection of multiple blood samples over periods of 6 to 12 hours to monitor serum levels of pituitary gonadotrophins during sexual maturation in the gilt. It was necessary that the technique be adaptable for gilts over a wide weight and age range, since cannulations were to be performed on the same pigs every 10 days, and that the technique not perturb gonadotrophin secretion. Sampling schedules were such that large diameter tubing was warranted to facilitate rapid withdrawal of blood. The technique developed, the short and long term effects, and the validation of the procedure are described below.

4.1.2. Catheterization Procedure

4.1.2.1. Animals

One hundred and eighty-six Yorkshire (Y), Landrace (L), Y x L, (Y x L) x Y and (Y x L) x L gilts were catheterized using this technique. Pigs ranged in age from 40 to 160 days and weighed between 30 and 115 kgs.

4.1.2.2. Procedure

Clear vinyl tubing (Dural Plastics and Engineering, Auburn, NSW, Australia, O.D. 1.50 mm, I.D. 1.00 mm), was cut in 60 cm lengths for gilts up to 60 kg and in 75 cm lengths for gilts over 60 kg. Eighteen gauge disposable needles (Monoject, St. Louis, MO., U.S.A.) were ground flat as
catheter to syringe adapters. Adapter plugs were made from 1 c.c. tuberculin syringes (Monoject, St. Louis, MO., U.S.A.) cut off at the 0.3 c.c. mark. Catheters, adapters, plugs and 14-gauge thin-walled reusable needles were soaked in a zepharin solution for one hour prior to use.

Gilts were caught and restrained using a snout snare. An elastic band was placed around the base of an ear to raise a vein. A 25-gauge butterfly infusion set (Terumo Corp., Japan) was inserted into the vein and saline flushed in, to verify the placement of the needle. Valium (Hoffmann-LaRoche Limited, Etobicoke, Ontario, Canada) diluted with an equal volume of sterilized saline, was injected, intravenously into an ear vein, at 0.5 mg/kg followed by 5 mg/kg ketamine (M.T.C. Pharmaceuticals, Cambridge, Ontario, Canada). The gilt was placed in dorsal recumbency with her forelegs held out to the sides (see Fig. 1). A 7.5 cm or 9 cm long 14-g thin-walled needle, fixed to a 12 c.c. syringe, half filled with saline, was inserted in the neck of the gilt approximately 2.5 cm anterior to and 2.5 cm lateral to the manubrium sterni. The needle was directed toward the midline at an angle of 30° (a greater angle as the animal increased in size) to both the median and frontal planes (see Figure 1). The needle was then drawn back slowly while gentle suction was applied to the syringe. When a vein was entered the syringe filled rapidly with blood. While holding the needle in position, the syringe was removed and 20 cm of a catheter was threaded through the needle into the vein. If resistance was encountered, the needle
was rotated but it was often more efficient to re-attach a syringe, containing fresh saline, and verify placement of the needle in a vein.

Once the catheter was in the vein, the 14-gauge needle was removed and an adapter and plug were fitted to the end of the tubing. The catheter was withdrawn until approximately 15 cm remained inside the gilt. The patency of the catheter was checked at this stage while an assistant bent the fore legs into the chest and pushed down. This mimicked a prostrate sleeping position, which put the most pressure on the catheters; small adjustments in the length of the catheter at this stage helped ensure good flow later, regardless of the gilt’s position.

A 4 cm square piece of surgical tape was folded over the catheter where it exited the neck. This flap of tape was glued to the gilt’s skin using Hip-Tag cement (Bigley Supply Co., Elyson, Minnesota). A 6 cm X 8 cm square of Elastoplast tape (Smith and Nephew, Lachine, Quebec, Canada) was glued on top of the surgical tape. The exposed end of the catheter was placed down the side of the pig’s neck and Elastoplast tape wound around the neck twice covering the catheter in the process. The end of the Elastoplast was glued down with cement. The catheter, buried under the tape, was accessible from the back of the neck.

Catheter maintenance consisted of flushing the catheters with 12 ml of saline twice daily. Heparin was not used in an effort to restrict the number of variables which may affect reproductive hormone secretion.
Additionally, to reduce the incidence of infection, a single 3 ml dose of Borgal (Hoechst Canada, Montreal) was administered intravenously to each pig within 6 hours of cannulation. During long-term cannulations, the same dose of Borgal was given daily until 48 hours prior to sampling. When catheters were removed, gilts were given 3ml of Borgal.

Removal of the catheters was accomplished by cutting the elastoplast tape behind the gilt’s neck and quickly pulling the tape and the catheter free. Catheterization appeared to have no long term consequences save some hemaetomas at the point of exteriorization. Healing of the point of exteriorization was complete within the 10 days between successive cannulations.
Figure 4.1: Site of puncture (diagram A), illustration of angle of puncture (diagram B), measurement of the length of catheter inside the gilt (diagram C) and illustration of the elastoplast patch and angle of exit of catheter (diagram D) during vena cava catheterization in the gilt. (Drawings by J. Duebner).
4.2. Effect of valium and ketamine on serum concentrations of LH and FSH.

4.2.1. Introduction

Valium and ketamine are injectable anaesthetics that are often used in combination for both their anaesthetic and analgesic properties. Valium and ketamine are often used in pigs prior to maintenance with halothane gas (Roberts, 1971). In swine, the combination of these two drugs, at the recommended doses of 0.55 mg/kg I.V. for valium and 11 mg/kg I.V., provides sufficient anaesthesia (15 to 35 minutes) to perform minor surgery and intubation (Wright, 1982).

Ketamine, 2-(0-chlorophenyl)-2-(methylamino) cyclohexanone, is water soluble, acidic and has a half life of approximately 1 hour in swine (Thurmon, 1986). It is absorbed quickly, due to its high lipid solubility, after either intramuscular or intravenous administration (Wright, 1982). Ketamine appears to depress the central nervous system without blocking sensory input at the spinal cord level. While input still reaches the cortical reception areas, perception is altered since the association areas are disorganized (Mori et al., 1971).

Metabolism of ketamine is accomplished by the liver in most species (Kaka et al., 1979). Initially, however, ketamine is redistributed from the blood to muscle and fat leading to the conclusion that the duration of
anaesthesia is a product of its half-life in the plasma (Wright, 1982). In cattle and human serum little protein binding of ketamine occurs, while in cats, horses and dogs up to 50% of the free ketamine is protein bound (Wright, 1982). No information is available on protein binding of ketamine in swine, but it is assumed that, like cattle, little occurs. Once in the liver, ketamine is converted to two primary metabolites, an N-methylated form and an oxidation product, both of which are subsequently excreted in the urine.

Valium, chemically called diazepam, is 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (Booth and McDonald, 1982). Valium is the most potent member of the family of benzodiazepines, drugs used as sedatives, muscle relaxants, and anti-convulsants. Benzodiazepines act as disinhibitors of suppressed behaviour in the reticular formation of the brainstem, thus producing a taming effect. Valium receptors are diffusely spread throughout the entire brain, except for the white matter (Booth and McDonald, 1982) suggesting that the suppressive effects of valium occur at many levels.

Interestingly, valium blocks polysynaptic, but not monosynaptic, reflexes (Booth and McDonald, 1982). Within the reflex arcs, valium acts together with many neurotransmitters, such as acetylcholine, serotonin, γ-aminobutyric acid (GABA) and dopamine to potentiate a GABA-mediated inhibition in the central nervous system (Booth and McDonald, 1982). Once
administered, valium binds to a receptor and activated an anion ionophore. This is followed by a decrease in the production of the cellular second messenger, cyclic guanosine 3'-5'-monophosphate (cGMP) and a decrease in cell activities requiring this messenger (Booth and McDonald, 1982).

The rapid onset and rapid disappearance of the disinhibitory effects of valium on the central nervous system are the two unique features of this drug (Booth and McDonald, 1982). Because of this, valium has been used extensively in clinical situations for behaviour modification, induction of anaesthesia and anti-convulsants (Booth and McDonald, 1982). However, valium is extremely irritating, both intravenously and intramuscularly. Other related, faster acting, less irritating and water soluble compounds such as midazolam maleate have been developed, but they are still not as widely used as is valium (Booth and McDonald, 1982).

When administered in humans, in combination with valium, ketamine induced anaesthesia was prolonged (Lo and Cumming, 1975). It has been postulated that this increase was due to an increased half-life of ketamine, probably due to competition with the valium for liver transformation systems. This would result in competitive inhibition of ketamine metabolism and, thus, prolonged action (Wright, 1982).

Valium is often administered in conjunction with ketamine in swine. When injected intravenously before ketamine it seems to reduce the
frequency of seizures often associated with ketamine (Rucker, 1976). The half-life of valium in swine is approximately 10 hours (Thurmon, 1986).

Both valium and ketamine have been implicated in the short-term alteration of reproductive hormone profiles. Ketamine has been shown to cause a depression in LH release in ovariectomized ewes (Clark and Doughton, 1983) and has been shown to both increase (Cohen et al., 1983) or cause a slight reduction (Matzen et al., 1987) in serum LH concentrations in rats. Treatment with valium can shift the circadian rhythm of hormone release; it has been shown to specifically alter the timing, but not the magnitude or duration, of LH surges in hamsters (Turek and Losee-Olson, 1988). However, no data is available regarding the effect of these two drugs on reproductive hormone release in gilts.

Given that valium and ketamine were used to anaesthetize the gilts prior to catheterization, it was necessary to evaluate the effects of the drugs on LH and FSH release characteristics. It was also necessary to determine how much time was required for any adverse effects of the procedure to disappear.

4.2.2. Methods

Four, 77 day old (Y X L) X Y gilts were purchased from the Prairie Swine Research Centre, Saskatoon, SK and transported to the WCVM.
When the gilts were 82 days old three blood samples were obtained by venipuncture at 0900, 1200 and 1500 hours to determine a pre-catheterization mean serum concentration of LH and FSH.

The following day, gilts were anaesthetized and catheterized. Intensive blood sampling began 15 minutes following anaesthetization and continued according to the schedule set out in Table 4.1.

Periods of intensive blood sampling were timed to correspond to the half-lives of the two drugs. The half-lives of valium and ketamine in the pig are estimated to be 10 and one hour, respectively (Thurmon, 1986). The sampling schedule, to determine the effects of these drugs on LH secretion parameters, was based on these times (see Table 4.1). The first intensive sampling period was intended to monitor both the initial effects of the drugs on the gilts as well as the changes in LH release during the first four half-lives of ketamine. The second and third intensive sampling periods, at 8 and 24 hours post-cannulation, were timed to follow the first two half-lives of valium. Forty-eight hours following the procedure a final intensive sampling period was conducted to determine if the drug combination had any residual effects.

In between hourly samples, and at the end of each day, catheters were flushed with 12ml of fresh saline. The dead-space of the catheter was then filled with 3ml of saline. Prior to sampling on the following day, the
catheter was flushed with 6ml of saline before the first blood sample was drawn.

Table 4.1: Intensive blood sampling schedule before (-24 hours) and after anaesthetization (0 - 4 hours) with valium and ketamine and catheterization and for 3 days following anaesthetization and catheterization (4 - 70 hours).

<table>
<thead>
<tr>
<th>Day and time1</th>
<th>Sampling Schedule</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday (-24 hours)</td>
<td>3 samples by venipuncture</td>
<td>to determine mean serum concentrations of LH and FSH</td>
</tr>
<tr>
<td>Tuesday (0-4 hours)</td>
<td>every 15 minutes for 4 hours post-anaesthetization and catheterization</td>
<td>first 4 half-lives of ketamine</td>
</tr>
<tr>
<td>(4-8 hours)</td>
<td>1 sample every hour for 4 hours</td>
<td>to determine mean serum concentrations of LH and FSH</td>
</tr>
<tr>
<td>(8-12 hours)</td>
<td>every 15 minutes for 4 hours</td>
<td>first half-life of valium</td>
</tr>
<tr>
<td>Wednesday (20-24 hours)</td>
<td>every 15 minutes for 4 hours</td>
<td>second half-life of valium</td>
</tr>
<tr>
<td>(24-28 hours)</td>
<td>1 sample every hour for 4 hours</td>
<td>to determine mean serum concentrations of LH and FSH</td>
</tr>
<tr>
<td>Thursday (44-48 hours)</td>
<td>every 15 minutes for 4 hours</td>
<td>third half-life of valium</td>
</tr>
<tr>
<td>(48-52 hours)</td>
<td>1 sample every hour for 4 hours</td>
<td>to determine mean serum concentrations of LH and FSH</td>
</tr>
<tr>
<td>Friday (66-70 hours)</td>
<td>1 sample every hour for 4 hours</td>
<td>to determine mean serum concentrations of LH and FSH</td>
</tr>
</tbody>
</table>

1. Time 0 represents the time of anaesthetization and catheterization.
Following the period of intensive blood collection the catheters were flushed daily with 12ml of saline and maintained for 15 days to determine the patency of the catheters.

4.2.3. Statistics

Analysis of data from the hourly blood collections and intense blood collections, yielded homogenous variances for LH pulse frequency data, as well as data for mean serum LH and basal LH concentrations (Bartlett's Tests: P < 0.6, P < 1.0, P < 0.6 respectively). However, variance for LH pulse amplitude data was heterogenous (P < 2 X 10^-6). LH pulse amplitude data, was inverse transformed prior to statistical analysis, as suggested by the Box-Cox procedure. All FSH data were homogenous for variance and thus no transformation was applied.

4.2.4. Effects of ketamine and valium on serum concentrations of LH and FSH

Analysis of variance showed that the combination of anaesthetization with ketamine and valium and the stress of the catheterization procedure had no effect on mean serum LH concentrations as derived from hourly samples or on the mean concentration of LH calculated by PULSAR from intensive samples (Table 4.2). Similarly, there was no effect of the
Table 4.2: Serum LH parameters in gilts sampled once per hour for LH hourly means or every 15 minutes for 4 hours for serum LH pulse amplitude, pulse frequency, mean and basal concentrations, before and after anaesthetization with valium and ketamine and catheterization¹.

<table>
<thead>
<tr>
<th>Time² (hours)</th>
<th>Hourly Mean³ ng ml⁻¹</th>
<th>Amplitude ng ml⁻¹</th>
<th>Frequency pulses hr⁻¹</th>
<th>Mean Conc. ng ml⁻¹</th>
<th>Basal Conc. ng ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>-24 hours</td>
<td>0.7 ± 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-4 hours</td>
<td></td>
<td>1.0 ± 0.09ᵃ</td>
<td>0.8 ± 0.1</td>
<td>0.5 ± 0.09</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td>4-8 hours</td>
<td>0.5 ± 0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-12 hours</td>
<td></td>
<td>1.4 ± 0.2ᵃ</td>
<td>0.9 ± 0.09</td>
<td>0.6 ± 0.1</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td>20-24 hours</td>
<td>2.0 ± 1.1</td>
<td>1.8 ± 0.4ᵇ</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.08</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>44-48 hours</td>
<td>0.6 ± 0.2</td>
<td>1.1 ± 0.1ᵃ</td>
<td>0.8 ± 0.3</td>
<td>0.5 ± 0.08</td>
<td>0.3 ± 0.06</td>
</tr>
<tr>
<td>66-70 hours</td>
<td>0.5 ± 0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Values are means ± S.E.M.; superscripts indicate differences within a column (P < 0.04).
² Time before and after catheterization.
³ Hourly mean was calculated as the average of 3 or 4 samples taken at hourly intervals.
Table 4.3: Serum FSH concentrations in gilts blood sampled once per hour for 3 or 4 hours for FSH hourly means and every 15 minutes for 4 hours for intensive mean serum FSH concentrations before and after anaesthetization with valium and ketamine and catheterization\(^1\).

<table>
<thead>
<tr>
<th>Time(^2) (hours)</th>
<th>Mean FSH (Hourly) ng ml(^{-1})</th>
<th>Mean FSH (Intensive) ng ml(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>-24 hours</td>
<td>N/A(^3)</td>
<td>N/A(^3)</td>
</tr>
<tr>
<td>0-4 hours</td>
<td>8.9 ± 0.8</td>
<td>8.4 ± 0.9</td>
</tr>
<tr>
<td>4-8 hours</td>
<td>8.9 ± 0.8</td>
<td>8.4 ± 0.9</td>
</tr>
<tr>
<td>8-12 hours</td>
<td>6.9 ± 0.8</td>
<td>6.9 ± 0.8</td>
</tr>
<tr>
<td>20-24 hours</td>
<td>5.7 ± 0.8</td>
<td>4.6 ± 2.0</td>
</tr>
<tr>
<td>44-48 hours</td>
<td>7.2 ± 0.8</td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td>66-70 hours</td>
<td>7.4 ± 0.8</td>
<td>7.4 ± 0.8</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± S.E.M.
\(^2\) Hours before and after anaesthetization and catheterization.
\(^3\) Insufficient serum for analysis.

combined drug/catheterization procedure on LH pulse frequency or basal serum LH concentrations.

LH pulse amplitude began to rise within 8 hours of anaesthetization and catheterization and was increased significantly (\(P < 0.003\)) 24 hours post-anaesthetization and catheterization. Forty-eight hours following anaesthetization and catheterization, LH pulse amplitude was similar to that seen 4 hours following anaesthetization and catheterization.
Neither the hourly mean of FSH in serum or mean serum FSH derived by PULSAR from the intensive blood samples was seemingly altered by the administration of valium and ketamine (Table 4.3).

4.2.5. Long term catheter patency

Catheters installed as detailed above were maintained for 15 days before removal. Catheters worked extremely well over a maximum of 72 hours with minimal care and flushing with saline and up to 14 days with twice daily flushing with fresh sterilized saline and a daily dose of 3ml of Borgal per gilt.

4.2.6. Contraindications of the catheterization procedure

Infrequently (5 of 186), a high pressure blood vessel, presumably an artery, was encountered when attempting to cannulate a vein. Insertion of a catheter into an artery in the neck could be accomplished with difficulty, but all such catheters were pushed out within the ensuing 6 hours.

A small percentage of young gilts were agile enough to scratch their necks and remove their catheters. If not removed in the initial 24 hours, the catheters generally remained in place for the duration of the experiment.

A variety of maladies including multifocal valvular endocarditis, suppurative arthritis, pneumonia or widespread infection with *Actinomyces*
*pyogenes* or *Staphlococcus aureus* occurred, within two weeks, in 20% of the first 30 gilts catheterized. The endocarditis was likely a result of insertion of the catheter too far into the superior vena cava where it rubbed on a heart valve or on the wall of the blood vessel near the heart.

As a result of this incidence of infection, intravenous injections of 3ml of Borgal through the catheter, immediately following completion of the procedure and upon completion of the experiment, were added to the catheter methodology. All doses of Borgal ceased 24 hours prior to any intensive blood sampling period to reduce any possibility of Borgal affecting LH or FSH secretion patterns. Upon adopting this protocol the incidence of health problems, from any source, was reduced to less than 10 percent overall.

4.2.7. Discussion

A combination of valium and ketamine was chosen since effective anaesthesia could be produced quickly with few side effects. This made the technique simple, economical and repeatable since no surgery suite was required. Although equipment was not sterilized, care was taken to ensure that catheters were clean and that needles were sterilized in zepharin between successive cannulations.

Additionally, the use of these drugs precluded the possibility of halothane-induced malignant hyperthermia (MH), an often fatal (Riebold,
1986) genetically linked disorder sometimes referred to as porcine stress syndrome (PSS; Steffey, 1986). Both the techniques of Karalus and Downey (1989) and Ford and Maurer (1978) require sterile surgical conditions and both use halothane to maintain sedation throughout the procedure. Our technique, was accomplished non-surgically, without halothane and could be performed, with some experience, in under 4 minutes per gilt. This makes the repeated cannulation of many animals possible under many experimental situations. Also, this procedure appeared to be a safe alternative to surgical implantation of cannulae.

The doses of valium and ketamine used were reported to produce a general anaesthesia for 15 to 35 minutes (Wright, 1982). In this study gilts became wobbly after infusion of valium, were recumbent following the ketamine, and remained completely anaesthetized for 4 to 8 minutes. Recovery to standing required a further 15 minutes.

Recovery from anaesthesia with ketamine has been reported to be associated with convulsions and coughing in horses and cats (Wright, 1982). Valium decreases the incidence of the muscle spasms (Butera et al., 1978). For this reason we administered valium prior to the ketamine. Most pigs recovered well from the procedure and were standing, eating and drinking within 30 minutes of anaesthesia. Some gilts exhibited minor convulsions and raspy coughing and 4 turned blue for approximately 1 minute during recovery. All gilts who were experiencing difficulty were monitored
continuous and were assisted in standing to facilitate breathing. All gilts recovered from these difficulties within 2 hours. No fatalities from these abnormal recoveries were recorded.

Unlike studies with ewes (Clark and Doughton, 1983) and rats (Cohen et al., 1983; Matzen et al., 1987), valium and ketamine did not produce changes in mean serum LH concentrations in gilts. The increase in LH pulse amplitude 24 hours following anaesthetization and catheterization was no longer evident 48 hours following the procedure, suggesting that the effects of valium and ketamine, and the stress of the catheterization procedure, on reproductive hormone secretion were transient. Although valium and ketamine alter the timing, but not the magnitude or duration, of LH surges in hamsters, (Turek and Losee-Olson, 1988), there was no way to evaluate the timing of the LH surges in our gilts since no method for repetitive sampling exists that does not involve extreme stress from repeated venipuncture or anaesthetization with halothane or valium and ketamine.

4.3. Summary

This non-surgical catheterization procedure provided a method to quickly catheterize swine, for short or medium term multiple blood sampling, with minimal perturbation of LH secretion. However, the contraindications associated with the technique suggest caution and careful
implementation. Post-procedure monitoring is crucial to identify and assist gilts in distress.

Antibiotics immediately following the procedure had no apparent effect on age at first estrus or weight gain when catheterized gilts were compared to non-catheterized gilts from the herd of origin. Use of antibiotics is crucial to decrease the number of gilts with fatal infections.

The procedure itself could be completed in 4 minutes per gilt and required a minimum of 1 and a maximum of 2 support staff.
5.0. Relationship between mean serum LH and age at first estrus in the prepubertal gilt

5.1 Introduction

To maintain maximum production in commercial swine operations, 25% of sows are replaced yearly due to routine culling after four to six litters. However, many gilts are slow to mature or do not show regular estrus cycles and subsequently prove to be infertile or of low productivity. To ensure adequate replacement stock, a producer must keep extra gilts. Early identification of gilts with a poor future reproductive capability, followed by culling at market weight, could result in some savings to the industry.

Research conducted to decipher the process of sexual maturation in the gilt has been inconclusive and has provided little practical information for the producer with respect to management of replacement gilts. Few relationships have been established between an animal’s reproductive endocrine status and its reproductive potential (Wise et al., 1981). During sexual maturation in the gilt there are marked changes in gonadotropin secretion and ovarian estrogen production (Lutz et al., 1984; Camous et al., 1985) and a negative relationship between luteinizing hormone concentrations at 55 days of age and age at puberty (Wise et al., 1981) has been demonstrated.
Several studies reported the many fluctuations in the gilts' reproductive hormone profile (Pelletier et al., 1981; Colenbrander et al., 1982; McNamara et al., 1985; Camous et al., 1985). Only the study by Camous and his colleagues (1985), however, reported a hormone pattern that appeared to be associated with sexual maturation in the gilt. Camous et al. (1985) describe a four stage increase in urinary estrone excretion prior to the expression of first estrus with each stage linked to the physical events of sexual maturation. The first stage, the perinatal phase, was characterized by high FSH and LH. The second phase, called the infancy period, occurred during the second month of age. In infancy LH is low, FSH concentrations are high and increase transiently at the end of the phase, and estrone levels are low. The third stage, occurred between three and four months of age, and was described as the initiation phase of sexual development. Here, increased FSH and LH stimulated development of the antral follicles and estrogen production from the ovaries. The fourth stage, between 5 months of age and puberty, was characterized by the final increase in estrogen production. These authors postulated that stages of different duration between gilts were responsible for the variation in age at first estrus in gilts (Camous et al., 1985). It is further possible that variable, defective or slow ovarian follicular growth in any of the four stages was responsible for the variation in age at first estrus and for the presence of approximately 25% of gilts which fail to exhibit normal estrous cycles.
Our objective was to determine if there was any relationship between serum concentrations of estradiol-17β, LH or FSH measured at various times during sexual development, and eventual age at first estrus. If so, with further research, a simple blood test for gilt reproductive potential could possibly be developed to identify which gilts will have a promising reproductive future and which gilts should be culled at market weight due to a postulated poor reproductive future.

5.2 Materials and Methods

5.2.1. Animals

Sixty-three Yorkshire (Y), Landrace (L), L x Y, or L x (L x Y) gilts were used in this study which was conducted at PSC. Three animals were removed from the study due to lameness. Of the three replicates conducted (n=21; n=21; n=18), the first and third were run from June to October (summer gilts) and the second from December to March (winter gilts).

For replicates 1 and 2, 10 ml blood samples were taken by jugular venipuncture twice daily, at 9 a.m. and 3 p.m., for two consecutive days starting at 60, 81, 102, 123 and 144 days of age. Gilts were restrained with a snout snare while blood samples were taken. For replicate 3, 10 ml blood samples were taken by jugular venipuncture twice daily for two consecutive
days starting at 60, 81 and 102 days of age for the purpose of increasing the number of animals at each of the first three ages.

At 135 days of age, gilts were moved to novel pens for continuous boar exposure and estrus detection until 200 days of age.

5.2.2. Radioimmunoassays

Intra- (n=9) and inter- (n=20) assay coefficients of variation (CVs) for LH were 9.2% and 10.7% respectively for a reference serum (mean=1.0 ng ml\(^{-1}\)) replicated in each assay. Intra- (n=10) and inter- (n=14) assay CVs for FSH were 10.5% and 7.3% respectively for a reference serum (mean=3.5 ng ml\(^{-1}\)). Intra- (n=18) and inter- (n=45) assay CVs for FSH were 8.1% and 3.0% respectively for a reference serum (mean=8.7 ng ml\(^{-1}\)). Estradiol concentrations for gilts in replicate 1 were analyzed in a single assay with an intraassay CV of 13.3%.

5.2.3. Statistics

The overall percentage of gilts exhibiting or failing to exhibit estrus before 200 days of age was compared between the summer (replicates 1 and 3 combined) and winter gilts (replicate 2) by Chi-Square analysis (SAS, Version 6.1).

Gilts were subdivided into groups in two different ways prior to statistical analysis. Gilts were first divided into two groups according to
whether or not they responded to boar introduction by exhibiting first estrus before 200 days of age.

Gilts were also divided into four groups according to the age at which they exhibited first estrus:

<table>
<thead>
<tr>
<th>Group</th>
<th>Age at First Estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>early estrus group</td>
<td>140 and 160 days of age</td>
</tr>
<tr>
<td>middle estrus group</td>
<td>160 and 180 days of age</td>
</tr>
<tr>
<td>late estrus group</td>
<td>180 and 200 days of age</td>
</tr>
<tr>
<td>non-responding gilts</td>
<td>&gt; 200 days of age</td>
</tr>
</tbody>
</table>

All LH data were log transformed to correct heterogeneity of variance. Analysis of variance for repeated measures, with replicate included as an independent variable, revealed that replicate had a significant effect ($P < 0.0001$) on the serum LH data. The replicate effect was no longer significant when replicate 2 data were removed from the analysis. LH data were then organized into two sets, summer gilts (replicates 1 and 3) and winter gilts (replicate 2), for analysis.

Each set of data were analyzed for group effect on mean serum LH concentrations as derived from the first blood sample at each age and as derived from the four blood samples taken over two days at each age.
Gilts were divided into two groups only (gilts which responded or failed to respond to boar exposure with first estrus) for FSH analysis since these data were the least representative of age at first estrus in the gilt. FSH data from replicates 1 and 3 were heterogenous for variance. Data from replicates 1 and 3 were ranked before statistical analysis as no appropriate transformation could confer homogeneity of variance. FSH data for replicate 2 were homogenous for variance (P < 0.125). FSH data from each of the three replicates were analyzed, and are reported, separately since the replicate effect was significant (P < 0.0001) regardless of which replicates were grouped together.

Gilts were divided into four groups according to age at first estrus for estradiol analysis as it was postulated, as a result of previously published work (Camous et al., 1985) that estradiol concentrations were the most likely to relate to sexual development and age at first estrus in the gilt.

5.3 Results

5.3.1. Percentage of gilts exhibiting first estrus

Thirty-five of thirty-nine summer gilts exhibited estrus before 200 days of age and four gilts (10%) failed to exhibit estrus following boar introduction. Fifteen of twenty-one winter gilts exhibited estrus before 200
days of age while 6 (29%) failed to exhibit estrus before 200 days of age. There was no significant difference between the percentages of gilts responding or failing to respond to boar exposure with first estrus between the two seasons, likely due to the small number of gilts in each group (Chi-square value = 1.777, P<0.2).

Overall, 17% of gilts (10 of 60) failed to exhibit estrus, following boar exposure, before 200 days of age.

5.3.2. Age and weight of summer and winter gilts at first estrus

Average age and weight at first estrus, for summer and winter gilts responding to boar exposure by exhibiting first estrus before 200 days of age, was 168 ± 14 days and 98 ± 12 kg.

Gilts in the early estrus group had an average age and weight of 153 ± 6 days and 85.3 ± 8.0 kg; gilts in the middle estrus group had an average age and weight of 168 ± 4 days and 100 ± 7.8 kg; gilts in the late estrus group had an average age and weight of 191 ± 8 days and 114.3 ± 10.9 kg; and gilts which failed to respond to boar exposure with first estrus were more than 200 days old and weighed more than 130 kg.

There was no significant difference in average age and weight at first estrus between replicates.
5.3.3. Mean serum LH concentrations in summer gilts

5.3.3.1. Analysis using only the first blood sample at each age

At 81 days of age, gilts which responded to boar introduction with first estrus before 200 days of age had mean serum LH concentrations more than double that seen in gilts which failed to respond to boar exposure before 200 days of age (Table 5.1). This difference in LH concentrations between groups was not seen at any other age studied (Table 5.1). Mean serum LH concentrations in gilts which responded to boar exposure with first estrus before 200 days of age peaked at 81 days of age, were lowest at 60, 102 and 123 days of age, and were intermediate at 144 days of age (Table 5.1). In gilts which failed to respond to boar exposure, mean serum LH concentrations did not differ significantly between 60 and 144 days of age; however, a small, but non-significant, increase in mean serum LH concentrations occurred at 102 days of age, 20 days later than the peak seen in gilts which responded to boar introduction (Table 5.1).

At 81 days of age, gilts which responded to boar exposure in the early estrus group had mean serum LH concentrations more than two-fold (P < 0.02) higher than the mean serum LH concentrations in gilts which failed to respond to boar exposure (Table 5.1). At 81 days of age, mean serum LH concentrations in gilts responding to boar exposure in either the middle or late estrus groups were intermediate to concentrations seen in the boar
Table 5.1: Mean serum luteinizing hormone concentrations (ng ml⁻¹) in summer gilts (replicates 1 and 3) sampled once at 60, 81, 102, 123 and 144 days of age. Responders showed estrus at the indicated ages while non-responders failed to show estrus by 200 days of age.

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Age at first estrus (days)</th>
<th>Age (days) blood sampling started</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
<td>81</td>
</tr>
<tr>
<td>Early responder (14)</td>
<td>140-160</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Middle responder (16)</td>
<td>160-180</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Late responder (5)</td>
<td>180-200</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Non-responder (4)</td>
<td>&gt;200 days</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Responder (35)</td>
<td>140-200</td>
<td>0.7 ± 0.1ᵃ</td>
</tr>
<tr>
<td>Non-responder (4)</td>
<td>&gt;200 days</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

¹. Values are means ± S.E.M.
Lower case superscripts indicate differences down a column; upper case superscripts indicate differences across a row.
exposed gilts in the early or non-responding groups (Table 5.1). There were no significant differences between groups at any other age studied (Table 5.1).

5.3.3.2. Analysis using four blood samples over two days at each age

When gilts were divided into responding and non-responding groups, gilts responding to boar introduction with first estrus had higher ($P < 0.008$) mean serum LH concentrations at 81 days of age than gilts which failed to show estrus before 200 days of age (Table 5.2). The highest mean serum LH concentrations in boar exposed gilts which responded with first estrus occurred at 81 and 102 days of age (Table 5.2). A similar significant trend of mean serum LH concentrations over time was not seen in boar exposed gilts which failed to show estrus (Table 5.2). Interestingly, mean serum LH concentrations in 102 and 123 day old non-responding gilts did appear to be increased compared to mean serum LH concentrations at 81 days of age. This increase, however, was not significant.

At 81 days of age, gilts responding to boar exposure in the early and late, but not middle, estrus groups had higher ($P < 0.0002; P < 0.003$ respectively) mean serum LH concentrations than gilts which failed to respond to boar introduction (Table 5.2). When mean serum LH data from gilts responding to boar exposure in the middle and late estrus groups were
Table 5.2: Mean serum luteinizing hormone concentrations (ng ml\(^{-1}\)) in summer gilts (replicates 1 and 3), blood sampled 4 times in two days, at 60, 81, 102, 123 and 144 days of age. Responders showed estrus at the indicated ages while non-responders failed to show estrus by 200 days of age\(^1\).

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Age at first estrus (days)</th>
<th>60</th>
<th>81</th>
<th>102</th>
<th>123</th>
<th>144</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early responder (14)</td>
<td>140-160</td>
<td>0.7 ± 0.1</td>
<td>1.6 ± 0.2(^a)</td>
<td>1.3 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Middle responder (16)</td>
<td>160-180</td>
<td>0.6 ± 0.1</td>
<td>1.1 ± 0.1(^\text{abc})</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Late responder (5)</td>
<td>180-200</td>
<td>0.6 ± 0.2</td>
<td>1.3 ± 0.2(^\text{ab})</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Non-responder (4)</td>
<td>&gt;200 days</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1(^b)</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Responder (35)</td>
<td>140-200</td>
<td>0.6 ± 0.1(^A)</td>
<td>1.3 ± 0.1(^\text{R}) (^A)</td>
<td>1.2 ± 0.1(^B)</td>
<td>0.9 ± 0.1(^\text{AC})</td>
<td>0.9 ± 0.1(^\text{AC})</td>
</tr>
<tr>
<td>Non-responder (4)</td>
<td>&gt;200 days</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1(^r)</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± S.E.M.

Lower case superscripts indicate differences down a column; uppercase superscripts indicate differences across a row.
combined into one group, the combined middle/late estrus group mean serum LH concentration was higher \((P < 0.003)\) than the mean serum LH concentration from gilts which failed to exhibit first estrus following boar introduction.

5.3.4. Mean serum LH concentrations in winter gilts

5.3.4.1. Analysis using only the first blood sample at each age

There was no significant difference in mean serum LH concentrations between gilts exposed to a boar and showing estrus before 200 days of age and gilts exposed to a boar and failing to responding with estrus before 200 days of age (Table 5.3).

5.3.4.2. Analysis using four blood samples at each age

When gilts were divided into two groups based on their response to boar introduction, there was no difference at any age studied, in mean serum LH concentrations between boar exposed gilts showing or not showing estrus (Table 5.4). Winter gilts responding to boar exposure with first estrus before 200 days of age showed a steady decline in mean serum LH concentrations between 60 and 144 days of age \((P < 0.0001)\) (Table 5.4). Winter gilts which failed to respond to boar exposure with estrus also showed an overall decline in mean serum LH concentrations between 60
Table 5.3: Mean serum luteinizing hormone concentrations (ng ml⁻¹) in winter gilts (replicate 2), blood sampled once starting at 60, 81, 102, 123 and 144 days of age. Responders showed estrus at the indicated ages while non-responders failed to show estrus by 200 days of age¹.

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Age at first estrus (days)</th>
<th>60</th>
<th>81</th>
<th>102</th>
<th>123</th>
<th>144</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early responder (4)</td>
<td>140-160</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Middle responder (10)</td>
<td>160-180</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Late responder (1)</td>
<td>180-200</td>
<td>0.3</td>
<td>0.7</td>
<td>0.4</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Non-responder (6)</td>
<td>&gt;200 days</td>
<td>0.8 ± 0.4</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Responder (15)</td>
<td>140-200</td>
<td>0.5 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Non-responder (6)</td>
<td>&gt;200 days</td>
<td>0.8 ± 0.4</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

¹ Values are means ± S.E.M.
Table 5.4: Mean serum luteinizing hormone concentrations (ng ml⁻¹) in winter gilts (replicate 2), blood sampled 4 times over 2 days starting at 60, 81, 102, 123 and 144 days of age. Responders showed estrus at the indicated ages while non-responders failed to show estrus by 200 days of age¹.

<table>
<thead>
<tr>
<th>Group ¹(N)</th>
<th>Age at first estrus (days)</th>
<th>60</th>
<th>81</th>
<th>102</th>
<th>123</th>
<th>144</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early responder (4)</td>
<td>140-160</td>
<td>0.4 ± 0.1⁵</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Middle responder (10)</td>
<td>160-180</td>
<td>0.7 ± 0.1⁵</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>Late responder (1)</td>
<td>180-200</td>
<td>0.7</td>
<td>0.7</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Non-responder (6)</td>
<td>&gt;200 days</td>
<td>0.7 ± 0.2⁵</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Responder (15)</td>
<td>140-200</td>
<td>0.6 ± 0.1⁴</td>
<td>0.5 ± 0.1²</td>
<td>0.5 ± 0.1²</td>
<td>0.3 ± 0.0³</td>
<td>0.2 ± 0.0³</td>
</tr>
<tr>
<td>Non-responder (6)</td>
<td>&gt;200 days</td>
<td>0.7 ± 0.2⁴</td>
<td>0.4 ± 0.1²</td>
<td>0.4 ± 0.1²</td>
<td>0.6 ± 0.1²</td>
<td>0.4 ± 0.1²</td>
</tr>
</tbody>
</table>

¹ Values are means ± S.E.M.
Lower case superscripts indicate differences down a column; uppercase superscripts indicate differences across a row.
and 144 days of age ($P < 0.001$)(Table 5.4). However, at 123 days of age, mean serum LH concentrations in boar exposed gilts showing no estrus rose ($P < 0.05$) transiently to a level similar to that seen at 60 days of age.

Sixty day old winter gilts responding to boar exposure in the early estrus group had lower ($P < 0.0001$) mean serum LH concentrations compared to 60 day old winter gilts responding to boar exposure in the middle, late or non-responding estrus groups (Table 5.4). There were no significant differences in mean serum LH concentrations between groups at any of the other ages.

No statistical comparison could be made involving the single gilt in the late estrus group due to lack of degrees of freedom for analysis of variance.

5.3.5. Mean serum FSH concentrations from four samples at each age

5.3.5.1. Summer gilts

Replicate 1 summer gilts which exhibited first estrus in response to boar exposure showed their highest mean serum FSH concentrations at 60 and 81 days of age and their lowest at 123 and 144 days of age (Figure 5.1). Mean serum FSH concentrations at 102 days of age were intermediate to the high and low values at the other ages ($P < 0.05$).
Figure 5.1: Serum follicle stimulating hormone (mean ± S.E.M.), based on four samples collected over 2 consecutive days, in summer gilts (replicate 1) blood sampled starting at 60, 81, 102, 123, and 144 days of age. Responders showed estrus before 200 days of age while non-responders failed to show estrus by 200 days of age. Lower case letters denote differences between groups while upper case letters denote differences between ages.
Replicate 1 gilts which failed to respond to boar exposure with first estrus showed no significant variation in mean serum FSH concentrations amongst the ages studied (Figure 5.1). Gilts which failed to respond to boar exposure had lower mean serum FSH concentrations (P < 0.0001) at 60, 81 and 102 days of age compared to the concentrations shown by boar exposed gilts which did respond (Figure 5.1). At 123 and 144 days of age, gilts which responded or failed to respond had similar mean serum FSH concentrations (Figure 5.1).

In replicate 1, but not replicate 3, summer gilts, mean serum FSH data showed a significant (P < 0.0001) group-age interaction.

Replicate 3 summer gilts had higher (P < 0.0001) mean serum FSH concentrations than the summer gilts in replicate 1. However, the overall trend of differences in mean FSH values between the ages was similar to that seen in replicate 1: boar exposed gilts which responded with first estrus had higher (P < 0.05) mean serum FSH concentrations at 60 and 81 days of age than at 102 days of age (Table 5.5). No statistical analysis of mean serum FSH concentrations between groups was possible due to the lack of degrees of freedom in the non-responder group (N=1).

5.3.5.2. Winter gilts

In both responding or non-responding gilts, mean serum FSH concentrations were highest (P < 0.0001) at 60 days of age (Figure 5.2).
Table 5.5: Serum follicle stimulating hormone concentrations (ng ml\(^{-1}\)), based on four blood samples taken over 2 consecutive days, in summer gilts (replicate 3) blood sampled starting at 60, 81, and 102 days of age. Responders showed estrus at the indicated ages while non-responders failed to show estrus by 200 days of age\(^1\).

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (days) blood sampling started</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Responder (17)</td>
<td>11.7 ± 0.5(^A)</td>
</tr>
<tr>
<td>Non-responder (1)</td>
<td>13.9</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± S.E.M. Upper case superscripts indicate differences across a row.

Mean serum FSH concentrations then dropped until 102 days of age in both groups of gilts (Figure 5.2). In gilts which responded to boar exposure by exhibiting first estrus, mean serum FSH concentrations stabilized at 123 days of age and remained at a similar level at 144 days of age (Figure 5.2). However, in gilts which failed to respond to boar exposure, mean serum FSH concentrations were at their lowest (P < 0.05) at 102 and 123 days of age. By 144 days of age, mean serum FSH concentrations in non-responding gilts were increased compared to the values reported at 102 and 123 days of age (Figure 5.2).

At 60 days of age, gilts which responded to boar exposure had higher (P < 0.05) mean serum FSH concentrations than gilts which failed to responded to boar introduction (Figure 5.2). By 144 days of age, the pattern
Figure 5.2: Serum follicle stimulating hormone (mean ± S.E.M.), based on four samples collected over 2 consecutive days, in winter gilts (replicate 2) blood sampled starting at 60, 81, 102, 123, and 144 days of age. Responders showed estrus before 200 days of age while non-responders failed to show estrus by 200 days of age. Lower case letters denote differences between groups while upper case letters denote differences between ages.
was reversed, with non-responding gilts having higher mean serum FSH concentrations than gilts which responded to boar exposure (Figure 5.2).

The FSH data for replicate 2 (winter gilts) showed a significant group-age interaction ($P < 0.0003$).

**5.3.6. Mean serum estradiol concentrations**

In 144 day old gilts exposed to a boar and responding with first estrus in the early estrus group ($P < 0.0001$), serum concentrations of estradiol were markedly higher when compared to those in 144 day old gilts in the middle, late or non-responding estrus groups (Figure 5.3). At all earlier ages, all gilts showed similar serum estradiol concentrations (Figure 5.3).

**5.3.7. Summer vs. Winter gilts**

Winter gilts (Figure 5.2) which failed to respond to boar exposure had higher ($P < 0.0001$) mean serum FSH concentrations at 60 and 81 days of age than did the replicate 1 gilts which responded to boar introduction with first estrus (Table 5.5). Gilts which responded or failed to respond to boar introduction had similar mean serum FSH concentrations at 102 days of age (Figure 5.2; Table 5.5).
Figure 5.3: Serum estradiol (mean ± S.E.M.), based on four samples collected over 2 consecutive days, in summer gilts (replicate 1) blood sampled starting at 60, 81, 102, 123, and 144 days of age. Early estrus gilts, middle estrus gilts, and late estrus gilts showed first estrus between 140-160 days of age, 160-180 days of age and 180-200 days of age respectively. Non-responders failed to show estrus by 200 days of age. Lower case letters denote differences between groups.
5.4. Discussion

5.4.1. Mean serum LH concentrations in summer prepubertal gilts

Mean serum LH concentrations from summer gilts reported in this study were similar to those reported elsewhere (Pelletier et al., 1981; Camous et al., 1985). The increase in mean serum LH concentration in gilts responding to boar exposure in this study was seen between 80 and 102 days of age (Tables 5.1 and 5.2). However, increases in mean serum LH values have been reported elsewhere to last for up to 40 days (Pelletier et al., 1981; Camous et al., 1985).

The results of this study show that 81 day old summer gilts have different mean serum LH concentrations depending on whether they eventually exhibit or fail to exhibit first estrus (Table 5.1 and 5.2). Summer gilts which responded to boar exposure in the early estrus group showed much higher mean serum LH concentrations at 81 days of age than did summer gilts which failed to respond to boar introduction. This, along with serum LH concentrations of less than 0.75 ng ml\(^{-1}\) in summer gilts which failed to respond to boar exposure, suggests that a mean serum LH concentration greater than 1 ng ml\(^{-1}\) at 81 days of age may be a requirement for sexual maturation in the summer gilt.

It has been postulated that a rise in LH at this early point in sexual development, followed by a sharp decrease, reflects hypothalamic and
pituitary maturation, stimulation of the gonads to produce steroids and the establishment of steroid negative feedback (Ramirez and McCann, 1963; Foster and Karsch, 1975; Cook and Rawlings, 1986). In our study, mean serum LH concentrations were highest in 81 day old summer gilts which responded to boar exposure. Tertiary follicles are reported to begin developing and producing estradiol at this age, if not earlier, (Oxender et al., 1979; Christenson et al., 1985). The combination of LH and estradiol seems to support the beginning of sexual maturation in the gilt.

The decrease in mean serum LH concentrations after 102 days of age seen in boar exposed gilts which exhibited first estrus, along with the earlier hypothesized development of the steroid producing tertiary follicles, suggested that negative steroid feedback was being established between 81 and 102 days of age in our gilts. These ages are similar to those reported in the Wise (1982) study where mean serum LH, FSH and estradiol concentrations were compared between intact and ovarietomized gilts. Wise concluded that negative steroid feedback of gonadotrophin secretion by ovarian steroids is established at about 100 days of age.

In our study, the lack of any increase in mean serum LH concentrations in 81 day old summer gilts which failed to exhibit estrus following boar introduction may be indicative of either a malfunction or inadequacy somewhere in the hypothalamic-pituitary-gonadal axis or perhaps caused by external stimuli, such as heat or humidity, which
inhibited normal sexual development in these gilts. The subsequent minor increase in mean serum LH concentrations in the non-responding gilts at 102 days of age (Table 5.1) may be too late to trigger further development in the reproductive tract.

Which component of the LH secretory profile is responsible for the increase in mean serum LH concentrations at 81 days of age in boar exposed gilts which respond with first estrus is not known. Alterations in LH pulse frequency, pulse amplitude, or in mean or basal secretion could each account for the increase. (For further investigation see Chapter 6).

The possibility that LH values in the prepubertal gilts were related to age at first estrus was described in 1981 (Wise et al., 1981). Wise reported a negative correlation between mean serum LH concentrations at 55 days of age and age at first estrus. There was no such correlation reported at 80 days of age, although LH concentrations did increase two-fold in ovariectomized, but not intact, gilts (Wise et al., 1981). In our study, there were no differences in mean serum LH concentrations amongst 60 day old gilts which responded or failed to respond to boar exposure with first estrus. However, the discrepancy in the age at which the correlation occurs between this study and the Wise study could be due to myriad differences including different genetic stock, different feeding and management strategies and different blood sampling regimes. However, we can conclude from both the Wise study and this study that a connection exists between
mean serum LH concentrations in early development and age at first estrus in prepubertal gilts.

5.4.1.1. Effect of blood sampling regime on the relationship between mean serum LH concentration and age at first estrus in summer gilts

At each age, all gilts in this study were blood sampled 4 times over two consecutive days. Mean serum LH concentrations as derived from all four samples or derived from the first sample only were compared between responder and non-responder groups at each of the ages studied. Additionally, comparisons were made at each age between gilts which responded to boar exposure in the early, middle, late or non-responder estrus groups, to determine the sampling schedule that would disclose the closest relationship between mean serum LH concentrations and age at first estrus.

Practically, single samples would be more cost efficient in any commercial test developed in the future and would also be less stressful for the pigs. However, single samples are likely less accurate as LH is secreted in a pulsatile manner. Thus single samples would confer much variability on the results as some gilts would be categorized based on a sample at the peak of an LH pulse and others at the nadir. Four samples over two days would increase the accuracy of the mean LH concentration obtained in that the average mean serum LH concentration per gilt would likely include
values from more than one part of the LH pulse. However, four samples in such as short time frame would be, and is, very stressful on the gilts and would be more costly and labour intensive for the commercial producer to obtain.

Results showed that, when summer gilts were divided into early, middle, late and non-responder estrus groups, mean serum LH concentrations derived from single blood samples taken at 81 days of age provide the clearest relationship between LH and eventual age at first estrus. Summer gilts responding to boar exposure in the early estrus group had most of the highest mean serum LH concentrations when compared to gilts in the middle and late estrus groups. The gilts which failed to exhibit estrus had most of the lowest mean serum LH concentrations (Table 5.1). A similar, though not identical, pattern was seen when LH concentrations were derived from 4 blood samples over 2 days (Table 5.2). Again gilts in the early estrus group had most of the highest mean serum LH concentrations but gilts responding with middle estrus had mean serum LH concentrations which were statistically no different than LH values in gilts which failed to respond with first estrus.

When gilts were divided into only two groups based on response or no response to boar exposure, mean serum LH concentrations derived from 4 samples over 2 days were different between the groups at 81 days of age (Table 5.2). Mean serum LH concentrations derived from only one sample
at 81 days of age did not differ between the two groups (Table 5.1). However, all boar exposed gilts which responded with first estrus had mean serum LH concentrations greater than 1 ng ml\(^{-1}\) at 81 days of age and all gilts which failed to respond to boar exposure with first estrus had mean serum LH concentrations of less than 0.75 ng ml\(^{-1}\) suggesting that, for practical purposes, the absolute value of mean serum LH concentration may be more important than the number of samples used to determine the concentration.

Thus, it appeared that mean LH concentration in either a single blood sample or 4 blood samples collected over 2 days could both be used to identify a relationship between the time of first estrus in 81 day old summer gilts. Further investigation regarding this relationship could possibly lead to a commercial test where gilts with LH concentrations of less than 0.75 ng ml\(^{-1}\) at 81 days of age could be marked for culling and marketing thereby reducing the number of surplus gilts in the replacement herd.

5.4.1.2. Effect of season on the relationship between serum LH concentrations and age at first estrus

Seasonal effects on reproduction in the gilt have been noted before (Ntunde et al., 1979; Christenson, 1981; Awotwi and Anderson, 1985; Christenson, 1986). Overcrowding of gilts has been shown to inhibit the
onset of puberty in summer gilts more than in winter gilts (Rampacek et al., 1981). Winter gilts show first estrus earlier (Christenson, 1981) and at a lighter weight (Margiogenis and Robinson, 1976) than summer gilts and have more advanced reproductive development, likely due to the lack of heat stress (Christenson, 1986).

While increases in photoperiod have been shown to have questionable benefits on ovulation rate (Ntunde et al., 1979), supplemental lighting did have a positive effect in hastening puberty in winter, but not spring, gilts (Diekman and Hoagland, 1983). Regimes as extreme as total light or total darkness have been shown to have no detrimental effect on growth, age at first estrus or subsequent estrous cycles (Awotwi and Anderson, 1985).

Winter gilts in our study presented lower mean serum LH concentrations than reported elsewhere (Pelletier et al., 1981; Camous et al., 1985). Winter gilts also showed less fluctuation in their lower mean serum LH concentrations than did the summer gilts. As well, winter gilts in the early estrus group had mean serum LH concentrations similar to those values exhibited by boar-exposed gilts in the middle, late or non-responding estrus groups. No consistent differences were noted between groups of gilts at any of the ages studied in contrast to the marked differences exhibited by summer gilts at 81 days of age.

However, the number of non-responding gilts throughout this study was limited in comparison with the numbers of responding gilts. The low
number of non-responding gilts in each replicate made it difficult to isolate statistical differences between the mean serum LH concentrations of the responding and non-responding gilts at any of the ages. Extensive replication is needed to increase the number of gilts in the non-responding estrus group both in the summer and winter. Larger numbers of animals in each group would assist in the establishment of a relationship between mean serum LH concentrations and eventual age at first estrus.

In contrast to the reported literature (Christenson, 1986), the hypothalamic-pituitary-gonadal axis in summer gilts in this study appeared to be more active than that in winter gilts as evidenced by the summer gilt’s high serum LH concentrations. However, there was no difference in the average age at first estrus between winter and summer gilts in our study. This also contrasts with reported literature which concludes that summer temperatures inhibit sexual development (Christenson, 1986), libido (Claus and Weiler, 1985), and estrous cycles (Claus and Weiler, 1985). Why our gilts appeared to be more reproductively active in the summer months is not known; daily temperatures in the 2 months before replicate #1 was begun (1988), often exceeded 30°C, temperatures which would be expected to induce a delay in reproductive development in the summer maturing gilts. However, the opposite result was obtained. This suggests that, however the ambient temperature affects sexual development, the season of maturation is an important confounding variable in the determination of any
relationship between mean serum LH concentrations and age at first estrus.

A further confounding variable encountered in this study was photoperiod. As outlined in Chapter 3, the gilts at the PSC were housed under minimal light conditions with little ambient light entering through the ventilation apparatus. Some incandescent lighting was provided for a maximum of one hour per day. The effect of photoperiod on age at first estrus in the gilt is equivocal. Complete darkness (Awotwi and Anderson, 1985) or excessive light (Ntunde et al., 1979; Diekman and Hoagland, 1983; Awotwi and Anderson, 1985) are both reported to have no effect on puberty, ovulation rate, or on LH and FSH concentrations. But, while photoperiods with lengthened light phases have been reported to hasten estrus in fall maturing gilts (Diekman and Hoagland, 1983) there were no indications of any difference in age at first estrus between fall or summer maturing gilts in this study.

However, the different photoperiods between the barn at the PSC and the IHU and B-Wing of the WCVM may explain the increased variability seen in the WCVM data and the lack of consistency between the data in this study and that in Chapters 6 and 8.

5.4.2. Mean serum FSH concentrations in prepubertal gilts

While LH secretion appeared steady except for the transient rise at 81 days of age, mean serum FSH concentrations decreased as gilts matured.
By 144 days of age, mean serum FSH concentrations were lower than those reported elsewhere in gilts of the same age (Diekman et al., 1983; Camous et al., 1985). Decreases in mean serum FSH concentrations as gilts age have been seen before by some (Wise, 1982; Camous et al., 1985) but not others (Colenbrander et al., 1982) but it is agreed that serum FSH concentrations fluctuate less than serum LH concentrations (Christenson et al., 1985).

With the exception of the one gilt in the third replicate, all gilts which failed to respond to boar exposure with first estrus had mean serum FSH concentrations, up to 102 days of age, that were lower than those exhibited by boar exposed gilts which responded with estrus. This suggests that non-responding gilts may have abnormally low serum FSH concentrations in addition to the absence of a transient increase in serum LH at 81 days of age. This combination of low LH and FSH concentrations could have resulted in abnormal reproductive maturation.

All three replicates of gilts showed different maximum serum concentrations of FSH prior to 144 days of age. Two of the replicates (#1 summer and #2 winter) showed significant group-age interactions indicating that the secretion patterns of FSH were different between boar exposed gilts responding with or without first estrus. This is further evidence that non-responding gilts have different hypothalamic-pituitary development than gilts which respond with first estrus.
Replicate 3 summer gilts showed mean serum FSH concentrations an order of magnitude higher than those seen in the other two replicates. However, the pattern of decrease in serum FSH as the gilts aged was consistent. Thus it may be the slow decrease in mean serum FSH concentrations, due to the establishment of the negative steroid feedback system (Christenson et al., 1985), that is required for normal maturation and not a specific threshold mean serum FSH concentration.

5.4.3. Mean serum estradiol concentrations in prepubertal gilts

A four stage increase in urinary estrone, beginning at 40 days of age and ending with display of first estrus at 230 days, occurs as the gilt matures (Camous et al., 1985). Camous and his colleagues postulated that either the relative metabolism of estradiol to estrone increases as the gilt matures or that the synthesis and secretion of estradiol increases over time resulting in an increase in metabolite secretion. However, in our study, step-wise increase in serum estradiol concentrations was not detected. Estradiol concentrations increased moderately in gilts in the early or middle estrus groups or remained constant in gilts in the late or non-responding estrus groups in response to boar exposure.

Mean serum estradiol concentrations did not increase in our gilts until 144 days of age when the boar exposed gilts which showed early estrus
were 10 days pre-estrus. Similar patterns of estradiol secretion have been reported elsewhere (Karlbom et al., 1982; Esbenshade et al., 1982).

Christenson concluded that the lack of correlation between the urinary estrone data (Camous et al., 1985) and studies involving mean serum estradiol concentrations (Elsaesser and Foxcroft, 1978; Wise, 1982) is due to estradiol assays which are too insensitive to detect changes in ovarian estradiol production (Christenson et al., 1983). The limit of sensitivity of the estradiol assay used in this study was 1.0 pg ml\(^{-1}\) and the majority of estradiol data in our study were between 1 and 3 pg ml\(^{-1}\), near the limit of sensitivity. This suggests that Christenson’s conclusion may be operative in this study. If so, it is equally clear that mean serum estradiol concentrations have a poor pre-pubertal relationship to first estrus in the gilt.

Alternatively, it may be more informative, in future, to measure a serum estradiol conjugate or an estrogen different from estradiol-17\(\beta\). However, until a more accurate estradiol-17\(\beta\) assay is developed, determination of a relationship between age at first estrus and hormone concentrations should focus on mean serum LH concentrations.

5.4.4. Conclusions

Thus it appears that mean serum LH concentrations at 81 days of age in the developing gilt may be related to future reproductive capability.
From a commercial standpoint, with much additional research, the rise in serum LH concentrations above 1 ng ml$^{-1}$ of serum in 81 day old gilts has the potential of being developed into a simple blood test to identify those gilts which will respond to boar exposure with first estrus before 200 days of age. The result would be an increase in the genetic potential of a swine herd. As an inexpensive lab test, this procedure could be easily performed as part of normal management procedures thereby allowing a producer to cull, at market weight, a high percentage of gilts which will fail to cycle before 200 days of age. The savings generated from efficient culling in the replacement gilt herd would far outweigh the cost of the test itself.

5.4.5. Future research proposals

There is much further research to complete before any commercial development of these findings can proceed. The difference in results between summer and winter gilts requires further investigation to determine what aspect of the gilt's hormone profile is affected by season and why our gilts react in apposition to most recorded in the literature. In our study, winter gilts exhibited, and failed to exhibit, first estrus in percentages equal to that seen in summer gilts. However, the mean serum LH concentrations in winter gilts did not follow the same pattern that was seen in the summer gilts. This could prove to be an important
contraindication in any investigation regarding a relationship between mean serum LH concentrations and age at first estrus.

All data from this study was generated from gilts originating from the same herd. An extensive field trial involving gilts of different breeds, and different geographical locations is required to determine the generality of the results. The herd of origin of the gilts used in this study is tightly line bred with only three source breeds. While no breed or breed cross effects were found in our data it could be that our results are specific to the PSC herd only. A field trial utilizing large numbers of gilts could test not only the generality of the results but could serve to increase the number of gilts in each of the responding and non-responding groups and thereby help to normalize the data. This increase in numbers will serve to enhance the validity of the data as well as the practicality of developing a commercial test applicable to a wide variety of Canadian producers.
6.0. Characterization of the early rise in serum LH concentrations

6.1. Introduction

Serum LH and FSH concentrations fluctuate throughout sexual development in the gilt. In prenatal gilts, the fetal anterior pituitary begins to secrete pulses of LH by 81 days of gestation (Ponzilius et al., 1986). At the same age, the pituitary also begins to secrete high serum levels of FSH (Colenbrander et al., 1982).

Within a month after birth, serum LH concentrations drop (Diekman et al., 1983; Camous et al., 1985) and stay low until gilts reach approximately 60 days of age while FSH concentrations peak at 54 days of age. LH values then rise and LH pulse amplitude, LH pulse frequency and mean serum LH concentrations peak between 80 and 133 days of age (Pelletier et al., 1981; Diekman et al., 1983; Camous et al., 1985).

After 133 days of age, LH and FSH concentrations in gilts drop again (Diekman et al., 1983). Puberty is marked by increasing mean serum LH and FSH values, an increased frequency of low amplitude LH pulses (Lutz et al., 1984) and an increase in serum estradiol concentrations (Diekman and Trout, 1983).

The previous suggestion of an increase in hormone secretion at 80 days of age in gilts, coupled with our results suggesting that mean serum LH concentrations differed at 80 days of age between gilts responding and
not responding to boar exposure with first estrus (see Chapter 5), suggested that further characterization of LH and FSH release around 80 days of age was warranted. It was postulated that at 80 days of age, one or more aspects of the LH pulse release profile changed markedly, and that this change occurred only in gilts which responded to boar exposure by exhibiting first estrus prior to 200 days of age. Identification of this change would further elucidate which particular components of the LH pulse release profile are the harbingers of future reproductive capability.

6.2. Materials and Methods

6.2.1. Animals

Two groups of Yorkshire gilts were transported to B-Wing of the W.C.V.M. at 34 and 28 days of age respectively. Two replicates (n=12 and n=6) were conducted one week apart.

6.2.2. Procedure

Gilts were catheterized at 60, 79 and 102 days of age. Blood samples were collected every 15 minutes for 8 hours when gilts were 62, 81 and 104 days of age. At 135 days of age, gilts were moved to the IHU, exposed to boars and checked daily for external signs of estrus until 200 days of age.
6.2.3. Radioimmunoassays

Intraassay (n=30 and n=24) CVs for LH were 1% and 3.6% respectively for reference sera (mean = 1.0 ng ml\(^{-1}\) and 9.2 ng ml\(^{-1}\) respectively) replicated in each assay. Interassay (n=35 and n=32) CVs were 1.2% and 3.8% respectively for reference sera (mean = 1.2 ng ml\(^{-1}\) and 9.6 ng ml\(^{-1}\)) replicated in each assay.

Intra- (n = 31) and interassay (n=42) CVs for FSH were 4.1% and 7.4% for reference sera (mean = 9.9 ng ml\(^{-1}\)) replicated in each assay.

6.2.4. Statistics

Gilts were divided into two groups according to whether they responded or failed to respond to boar exposure with first estrus before 200 days of age.

LH pulse frequency data were transformed to the power -0.8 to correct heterogeneity of variance. Mean serum LH data also exhibited non-homogenous variances and were subjected to the rank procedure as no suitable transformation was possible. Serum FSH data, basal serum LH values and LH pulse amplitude data all exhibited homogeneity of variance and no transformations were applied.

Data were analyzed by two-way analysis of variance with replicate as an independent variable. Where replicate was found to have a significant
effect (LH pulse amplitude data and mean serum LH data) data from each replicate were analyzed separately.

6.3. Results

6.3.1. Age at first estrus and mean estrus interval

Twelve of the 18 gilts exposed to boars exhibited first estrus at 146 ± 0.6 days while the remaining 6 gilts all failed to exhibit first estrus before 200 days of age (P < 0.05). Of the gilts exhibiting first estrus, the mean first estrus interval was 21 ± 2.6 days.

6.3.2. Serum LH profile

In replicate 1, LH pulse amplitude was no different (Table 6.1) in gilts responding to boar introduction by showing first estrus compared to gilts which failed to respond to boar exposure (first estrus >200 days of age). Gilts in the second replicate had LH pulse amplitude values approximately twice that seen in gilts from the first replicate. However, as in replicate 1, there was no difference in LH pulse amplitude between gilts responding, or not responding, to the boar (Table 6.1).

Mean serum LH concentrations in replicate 1 rose significantly (P < 0.05) between 81 and 104 days in gilts responding and failing to respond to boar introduction with first estrus (Table 6.1). There was no change in
Table 6.1: Luteinizing hormone (LH) pulse amplitude (ng ml\(^{-1}\)) and mean serum LH concentrations (ng ml\(^{-1}\)) in gilts blood sampled every 15 minutes for 8 hours at 62, 81 and 104 days of age\(^1\). Responders showed estrus prior to 200 days of age while non-responders had not shown estrus by 200 days of age.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Group</th>
<th>62 days</th>
<th>81 days</th>
<th>104 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Responder (7)</td>
<td>Amplitude</td>
<td>0.6 ± 0.08</td>
<td>0.7 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>0.2 ± 0.03(^a)</td>
<td>0.3 ± 0.03(^a)</td>
</tr>
<tr>
<td></td>
<td>Non-responder (5)</td>
<td>Amplitude</td>
<td>0.6 ± 0.05</td>
<td>0.7 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>0.3 ± 0.02(^a)</td>
<td>0.3 ± 0.02(^a)</td>
</tr>
<tr>
<td>2</td>
<td>Responder (5)</td>
<td>Amplitude</td>
<td>1.6 ± 0.3</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>0.8 ± 0.3</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Non-responder (1)</td>
<td>Amplitude</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>0.4</td>
<td>N/A(^2)</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± S.E.M. Lower case superscripts indicate differences (P < 0.05) across the row.

\(^2\) No serum for analysis as gilt removed catheter before sampling commenced.
mean serum LH concentrations in either group between 62 and 81 days of age. There was also no difference in mean serum LH concentrations between gilts responding and not responding to boar exposure with estrus at any of the ages studied (Table 6.1).

In replicate 2, the low number of gilts which did not respond to boar introduction with estrus made statistical comparisons of mean serum LH concentrations invalid. There appeared to be no age effect on mean serum LH concentrations in either gilts responding or not responding to boar exposure with estrus (Table 6.1). Comparison of mean serum LH concentrations between groups was not possible.

At 62 and 81 days of age, gilts responding or failing to respond to boar exposure in replicate 2 had mean serum LH concentrations higher than those seen in gilts in replicate 1. By 104 days of age, however, all gilts in both replicates showed similar mean serum LH concentrations (Table 6.1).

LH pulse frequency and basal serum LH concentrations were similar in all gilts responding and not responding to boar exposure with estrus (Table 6.2). In addition, there was no effect of age on mean or basal serum LH concentrations in either gilts which responded or failed to respond to boar exposure with first estrus (Table 6.2).
Table 6.2: Luteinizing hormone (LH) pulse frequency (pulses hr⁻¹) and basal serum LH concentrations (ng ml⁻¹) in gilts blood sampled every 15 minutes for 8 hours at 62, 81 and 104 days of age¹. Responders showed estrus prior to 200 days of age while non-responders had not shown estrus before 200 days of age.

<table>
<thead>
<tr>
<th>Group</th>
<th>62 days</th>
<th>81 days</th>
<th>104 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Responder (12)</strong></td>
<td>Frequency</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Basal</td>
<td>0.2 ± 0.07</td>
<td>0.2 ± 0.04</td>
</tr>
<tr>
<td><strong>Non-responders (6)</strong></td>
<td>Frequency</td>
<td>0.5 ± 0.07</td>
<td>0.5 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Basal</td>
<td>0.1 ± 0.01</td>
<td>0.2 ± 0.01</td>
</tr>
</tbody>
</table>

¹ Values are means ± S.E.M.

6.3.3. Serum FSH

Two-way analysis of variance revealed no significant differences in mean serum FSH concentrations between gilts responding or not responding to boar introduction at any of the ages studied (Table 6.3); nor was there an effect of age on serum FSH concentrations in either responding or non-responding gilts (Table 6.3).

6.4. Discussion

The age at first estrus in gilts exposed to a boar and showing estrus was similar to that published elsewhere (Kirkwood and Hughes, 1979; Paterson and Lindsay, 1980; Eastham et al., 1984; Eastham and Cole,
1987). However, the range of ages was very small (20 days) when compared to gilts in the rest of the experiments combined (50 days). This could have been due to the fact that this experiment involved only purebred gilts whereas two-way and three-way breed crosses were used, in addition to purebred gilts, in all other experiments in this study.

Table 6.3: Mean serum follicle stimulating hormone concentrations (ng ml\(^{-1}\)) in gilts blood sampled every 15 minutes for 8 hours at 62, 81 and 104 days of age\(^1\). Responders showed estrus prior to 200 days of age while non-responders had not shown estrus before 200 days of age.

<table>
<thead>
<tr>
<th>Group</th>
<th>62 days</th>
<th>81 days</th>
<th>104 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders (12)</td>
<td>8.2 ± 0.7</td>
<td>8.5 ± 0.6</td>
<td>5.9 ± 1.3</td>
</tr>
<tr>
<td>Non-responders (6)</td>
<td>6.6 ± 0.5</td>
<td>7.5 ± 0.6</td>
<td>6.6 ± 1.6</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± S.E.M.

There were no dramatic changes in either serum LH pulse amplitude, LH pulse frequency, basal LH concentrations or mean serum FSH concentrations between the ages of 62 and 104 days in this study. This is in direct contrast to previously published results (Pelletier et al., 1981; Diekman et al., 1983; Camous et al., 1985) all of which reported changes at, or around, 80 days of age. In these previously published studies methods such as surgical installation of jugular catheters under general anaesthetic (Camous et al., 1985), jugular puncture (Colenbrander et al., 1977) and
jugular cannula insertion without anaesthetic (Diekman et al., 1983) were used to obtain blood samples. Our method of catheter placement, antibiotic treatment and stabilization period appeared to be no more stressful or invasive than those listed above, so it is unlikely that methodology interfered with the results obtained.

Mean serum LH concentrations did show a two-fold increase at 104 days of age when compared to mean serum LH concentrations at 62 and 81 days of age. This could suggest that the blood sampling periods allowed for in this study were completed too early and that a further intensive sampling period should have been conducted at 120 days of age.

The lack of any changes, save for an increase in mean serum LH concentrations at 104 days of age, also failed to elucidate what part of the LH secretory profile was altered at 80 days in gilts from the study investigating the prediction of first estrus using mean serum LH concentrations described in Chapter 5. Blood sampling in both this and the Chapter 5 study (replicate #2) occurred when the gilts were the same age and at the same time of the year. However, the absolute values for mean serum LH were different, although very slightly, between the two studies. At 62 days of age, Chapter 5 winter gilts showed mean serum LH concentrations of between 0.3 - 0.8 ng ml\(^{-1}\) of serum whereas gilts in this study had mean serum LH concentrations below 0.4 ng ml\(^{-1}\). At 81 days of age, responding and non-responding gilts from this study had similar mean
serum LH concentrations while responding winter gilts from the Chapter 5 study had mean serum LH concentrations more than double those seen in the non-responding winter gilts.

Additionally, LH pulse frequency and basal LH concentrations in gilts in this study were virtually identical to those shown by control gilts in the LH treatment study (see Chapter 8). However, the control gilts in the LH treatment study showed mean serum LH concentrations three to four times greater than the mean serum LH values from gilts in this study. The control gilts in the LH treatment study were assessed in the winter further indicating that all of the summer gilts in this study appeared to be exhibiting LH values more consistent with winter gilts.

However, the discrepancy in mean serum LH concentrations between this study and the Chapter 5 and Chapter 8 studies is puzzling. Given that the gilts in this study were purchased from the same herd as those in Chapters 5 and 8, it is difficult to explain why both sets of gilts failed to show similar changes in their hormone profiles at 81 days of age. Gilts in this study were reared as closely as possible to the gilts in the source herd and the gilts in the other studies. Gilts in this study were subjected to a stringent catheterization schedule at a young age and it is possible that the increased stress impacted on these gilts' ability to respond with normal hormonal changes in response to boar exposure. Interference of cortisol on the hormone profile of the gilt was seen in other boar exposed gilts which
failed to show estrus before 200 days of age (see Chapter 7). Indeed, in this current study (Chapter 6), 50% of gilts failed to show estrus. This is much higher than the average 25% seen in the source herd and the remainder of gilts in all of these studies combined.

The stress invoked in the prediction study was likely also very high, but it lasted for a very short time and was directly associated with the immediate procedure of blood sampling. The stress in this study, however, while perhaps identical at the initiation of each catheterization period to the stress in the prediction study, included the stress associated with the presence of the catheters as well as the subsequent confinement. This may have produced a heightened initial awareness in the gilts and chronic stress throughout the experiment.

6.5. Conclusion

The protocol in this study was inadequate to determine which component of pulsatile LH release was altered in 81 day old gilts which eventually responded to boar exposure with estrus before 200 days. It is possible that the stress associated with multiple catheterizations between 60 and 100 days is sufficient to perturb the normal hormonal development occurring at this age. Perturbation of hormonal changes at this age could then result in an increased incidence of non-responding gilts such as was seen in this study.
6.6. Future research proposals

A catheterization technique involving simple maintenance of catheters for at least 30 days, without health problems, is necessary before a study such as this could be attempted with the confidence of obtaining more useful data.

Repetition of this study should also include data from an age earlier than 60 days and later than 104 days in order to fully evaluate the gilts’ changing hormone profile throughout sexual maturity.
7.0. The effect of boar exposure on circulating concentrations of LH, FSH, cortisol and estradiol

7.1. Introduction

In several species, introduction of a male of the same species results in the induction of first estrus in peri-pubertal females (Brooks and Cole, 1970; Martin et al., 1978; Fadem, 1989). Exposure to a mature intact boar is one of the most effective means of stimulating early estrus in the gilt (Brooks and Cole, 1970). The majority of information about the "boar effect" consists of managerial details advocating the most effective exposure regimes to produce the earliest estrus.

Raising gilts from birth through maturity in the presence of intact or castrated males can be inconsequential in terms of age at first estrus (Eastham et al., 1984) or can delay puberty (Cole et al., 1982). This delay is due possibly to the gilts being too immature to respond to boar stimulation or the gilts becoming habituated to the boar stimulus and therefore unable to respond at a later age (Kirkwood and Hughes, 1982). However, habituation to males in general is unlikely, since gilts exposed continuously to one boar respond to novel boar exposure in the same way as gilts never before exposed to a male (Eastham and Cole, 1987).
Gilts are generally reared, isolated from males, until they reach an optimum age range between 135 and 170 days of age (Hughes and Cole, 1976; Kirkwood and Hughes, 1979). Then, they are either moved on a daily basis to the boar's pen for exposure or they can be permanently relocated to a novel pen adjacent to the boar's pen. A daily minimum of 30 minutes of boar exposure is sufficient to procure the "boar effect" on the timing of first estrus in the gilt (van Lunen and Aherne, 1987).

The age of the boar used is an important component of the "boar effect". Only mature males of at least 11 months of age are capable of producing the effect (Kirkwood and Hughes, 1980b; Kirkwood and Hughes, 1981). It has been postulated that males of less than 6 months of age are unable to synthesize or release the C19 pheromonal steroids implicated in the olfactory portion of boar stimulation (Kirkwood and Hughes, 1981). However, young boars can stimulate first estrus in gilts, given sufficient time (probably for the male to mature). Ovulation rate at first estrus in gilts exposed to young males is no different than that seen following stimulation by a mature male (Kirkwood and Hughes, 1981) suggesting that no detrimental effects on fertility are produced by extended exposure to immature boars.

The mechanism of the boar effect is still a mystery. What is known is that removal of the olfactory bulbs of prepubertal gilts, renders the animals insensitive to boar stimulation and results in bulbectomized gilts
showing estrus at the same time as control gilts not exposed to a boar (Kirkwood et al., 1981). This suggests that a major part of the female’s receptivity system is olfactory in nature. The stress of moving gilts to novel surroundings is synergistic with the effect of boar exposure on age at first estrus (van Lunen and Aherne, 1987) but stress itself is ineffective at inducing puberty (Pearce and Hughes, 1985). Recently, Pearce and Hughes postulated that stress, generated by full physical contact with a mature boar, was reflected by a substantial rise in plasma cortisol levels. This increase in cortisol resulted in an increase in LH secretion (Pearce and Hughes, 1987). More recently, Pearce and Paterson (1992) concluded that increased cortisol was not a factor in boar induced first estrus in the gilt, but full facial contact between boar and gilt was critical.

Exposure of gilts to 3α-androstenediol, a steroid in boar saliva, has been shown to induce early estrus (Kirkwood and Hughes, 1983) but the steroid effect could not be separated from the transport stress effect so no causal relationship could be inferred.

Thus, while several aspects of boar stimulation of first estrus in the gilt are under investigation, it is still not known what effect, if any, males have on serum concentrations of reproductive hormones in gilts. Theoretically, it is feasible to assume that exposure to boars results in either short term or long term alterations in the hormone profile of gilts which respond to the stimulation.
However, in any commercial management system, the usual practice of exposing gilts to mature boars to induce first estrus by 200 days of age is ineffective in a small number of pigs. Why some animals should be unresponsive while others respond is not known. It is logical to assume that gilts which fail to respond to boar stimulation present different hormonal alterations following boar exposure than those postulated to occur in responding gilts.

This study examined LH pulse amplitude, LH pulse frequency, mean serum and basal LH concentrations, as well as mean serum FSH, cortisol and estradiol concentrations, in gilts exposed to boars in contrast to gilts raised without boar exposure. Also, the hormone profiles between boar exposed gilts that exhibited first estrus (responders, R) or no estrus (non-responders, NR) before 200 days of age were compared. The objective was to determine if boar induced first estrus involves changes at the level of the hypothalamo-pituitary axis and if it does, what aspect of hormone release is stimulated in responding versus non-responding gilts.

7.2. Materials and Methods

7.2.1. Animals

The study was run in three replicates (n=8, 8 and 7 respectively). One animal was removed due to lameness.
Twenty-three Y X (L X Y) gilts were moved to B-Wing in the WCVM at 128 days of age and housed in groups of 7 or 8. Control gilts were housed in individual pens (1.5m X 2.5m, 12L/12D) in B-Wing. At 135 days of age, gilts were moved to and housed in individual pens (1.5m X 1.5m) adjacent to pens containing mature boar of at least two years of age. At 135 days of age, control gilts were rotated to novel pens within the same B-Wing housing room to simulate transport stress.

7.2.2. Experimental Protocol

Gilts were catheterized at 133 days of age. Forty-eight hours later, at T = 0 hours, a 6 hour intensive blood sampling period was conducted in which samples were collected from each gilt every 15 minutes. At T = 6 hours, gilts were moved to the IHU where they were transferred into the individual exposure pens for boar exposure. Concurrently, the control gilts were rotated between pens, in B-Wing, to produce an equivalent transport stress and novel surroundings. Blood samples were collected from all gilts every 15 minutes for a further 6 hours. Gilts were re-catheterized and two additional 8 hour intensive blood sampling periods conducted following 10 and 20 days of continuous boar exposure. Gilts were monitored daily for estrus until 200 days of age.
7.2.3. Radioimmunoassays

Intra- (n=9) and inter- (n=20) assay coefficients of variation for LH were 9.2% and 10.7% respectively for a reference serum (mean = 1.0 ng ml\(^{-1}\)) replicated in each assay. Intra- and interassay coefficients of variation were 8.3% (n=18) and 2.6% (n=67) or 8.9% (n=18) and 5.3% (n=52) for reference sera with mean FSH concentrations of 7.9 ng ml\(^{-1}\) or 11.7 ng ml\(^{-1}\), respectively.

Intra- and interassay coefficients of variation for estradiol concentrations were 22.2% and 8.2% or 15.2% and 2.1% for porcine reference sera with means of 5.4 pg ml\(^{-1}\) and 16.3 pg ml\(^{-1}\), respectively.

Estradiol concentrations were determined for each gilt from serum pools corresponding to each of the 4 sampling times: before and after initial boar exposure and then 10 and 20 days following continuous exposure.

7.2.4. Statistics

Gilts were subdivided into groups in two different ways. Gilts were divided into four groups according to the age at which they exhibited first estrus: early group gilts exhibited estrus between 140 and 160 days of age, middle group gilts exhibited estrus between 160 and 180 days of age, late group gilts exhibited estrus between 180 and 200 days of age and non-responding gilts failed to exhibit estrus before 200 days of age. Gilts were
of age ($P < 0.05$). The remaining 5 boar exposed gilts did not show estrus by 200 days of age.

### 7.3.2. Effect of boar exposure on LH

LH pulse frequency rose two-fold in the 6 hours immediately after boar introduction (see Figure 7.1 for representative animals) in gilts that showed estrus prior to 200 days of age (Table 7.1)($P < 0.001$). LH pulse frequency dropped somewhat, 10 days after initial exposure, reaching concentrations similar to those seen before boar introduction by 20 days of continuous boar exposure. Following 10 days of continuous boar exposure responding gilts had LH pulse frequencies that were intermediate compared to those in control and non-responding gilts (Table 7.1).

LH pulse amplitude did not change after boar introduction, but when LH pulse amplitude data were combined over all 20 days of the experiment it was found that gilts responding to boar introduction with estrus had LH pulse amplitudes intermediate between those seen in the control gilts and gilts not responding to boar exposure (Table 7.1).

Immediately after boar introduction, mean and basal concentrations of LH rose ($P < 0.004$ and $P < 0.01$ respectively) in gilts which eventually exhibited first estrus. After 10 days of continuous boar exposure, mean and basal LH concentrations were no different than those seen prior to boar exposure (Table 7.1).
Table 7.1: Luteinizing hormone (LH) pulse frequency (pulses hr⁻¹) and amplitude (ng ml⁻¹), and mean (ng ml⁻¹) and basal concentrations (ng ml⁻¹) of LH in serum, in gilts blood sampled every 15 min for 6 h immediately before and immediately after boar introduction at 135 days of age and following 10 and 20 days of continuous boar exposure₁ or transportation to a new pen only (controls). Responders showed estrus prior to 200 days of age, non responders had not shown estrus by 200 days of age.

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>10 days</th>
<th>20 days</th>
<th>Average²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>0.31 ± 0.08</td>
<td>0.31 ± 0.03</td>
<td>0.50 ± 0.10</td>
<td>0.34 ± 0.09</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>Amplitude</td>
<td>1.17 ± 0.23</td>
<td>1.72 ± 0.28</td>
<td>1.24 ± 0.15</td>
<td>1.12 ± 0.17</td>
<td>1.29 ± 0.1²</td>
</tr>
<tr>
<td>Mean</td>
<td>0.35 ± 0.07</td>
<td>0.46 ± 0.09</td>
<td>0.42 ± 0.07</td>
<td>0.34 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.18 ± 0.03²</td>
<td>0.22 ± 0.03</td>
<td>0.18 ± 0.03</td>
<td>0.15 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Non-responders (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>0.31 ± 0.10</td>
<td>0.50 ± 0.14</td>
<td>0.35 ± 0.10²</td>
<td>0.42 ± 0.10</td>
<td>0.40 ± 0.05</td>
</tr>
<tr>
<td>Amplitude</td>
<td>0.77 ± 0.10</td>
<td>1.00 ± 0.10</td>
<td>0.76 ± 0.10</td>
<td>0.93 ± 0.08</td>
<td>0.87 ± 0.05²</td>
</tr>
<tr>
<td>Mean</td>
<td>0.26 ± 0.04</td>
<td>0.42 ± 0.06</td>
<td>0.29 ± 0.03</td>
<td>0.37 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.13 ± 0.02²</td>
<td>0.20 ± 0.03</td>
<td>0.14 ± 0.02</td>
<td>0.25 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Responders (12)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>0.26 ± 0.05²</td>
<td>0.57 ± 0.08²</td>
<td>0.44 ± 0.07²CD</td>
<td>0.29 ± 0.06²AD</td>
<td>0.39 ± 0.04</td>
</tr>
<tr>
<td>Amplitude</td>
<td>1.07 ± 0.16</td>
<td>1.08 ± 0.11</td>
<td>0.94 ± 0.11</td>
<td>1.01 ± 0.07</td>
<td>1.05 ± 0.07²b</td>
</tr>
<tr>
<td>Mean</td>
<td>0.22 ± 0.03²</td>
<td>0.31 ± 0.03²</td>
<td>0.22 ± 0.03²AB</td>
<td>0.32 ± 0.04²AB</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.13 ± 0.01²</td>
<td>0.15 ± 0.03²</td>
<td>0.14 ± 0.02²AB</td>
<td>0.19 ± 0.04²AB</td>
<td></td>
</tr>
</tbody>
</table>

₁ Values are means ± S.E.M.
Lower case superscripts indicate differences down a column while the upper case superscripts indicate differences across a row.
² Mean LH pulse frequency and amplitude over all 20 days of exposure.
Figure 7.1: Representative mean serum LH (mean ± S.E.M.) profiles in a gilt not exposed to a boar (control), a gilt exposed to a boar but failing to exhibit first estrus before 200 days of age (non-responder), and in a gilt exposed to a boar and exhibiting first estrus before 200 days of age (responder). Arrows indicate the time of introduction to a mature boar (responders and non-responders) or, in the control gilts, the time of transport to a novel pen. Blood samples were taken every 15 minutes from vena catheters inserted 48 hours previously.
Before boar exposure, basal LH concentrations were similar in both boar exposed groups of gilts, but control gilts had higher basal LH concentrations compared to gilts responding to boar exposure with estrus ($P < 0.05$)(Table 7.1).

### 7.3.3. Effect of boar exposure on FSH

When data were summarized for all periods of observation, mean serum concentrations of FSH were higher in control gilts than those exposed to the boars ($P < 0.05$)(Figure 7.2). At 10 days after boar introduction, gilts that did not respond to boars with early estrus had lower mean serum concentrations of FSH than those gilts that responded with early estrus; control gilts had the highest concentrations of FSH. At 20 days after boar exposure, gilts that did not respond to boar exposure with estrus had mean serum concentrations of FSH that were intermediate to the control gilts and gilts responding to the boar with estrus. The boar exposed gilts not showing estrus experienced a transient decrease in mean serum concentrations of FSH after 10 days of boar exposure.

### 7.3.4. Effect of boar exposure on cortisol

Serum cortisol levels dropped in all gilts throughout the course of the period of initial boar introduction or repenning (control gilts)(Figure 7.3). When cortisol levels were combined within groups it was found that boar
**Figure 7.2:** Mean serum follicle stimulating hormone (mean ± S.E.M.) in gilts sampled every 15 minutes for 6 hours immediately before and after boar introduction at 135 days of age, as well as 10 and 20 days following continuous exposure or transportation to a new pen only (controls). Average category denotes mean serum FSH concentration overall all 20 days for each group. Responders showed estrus prior to 200 days of age while non-responders failed to show estrus before 200 days of age. Lower case letters denote differences between groups while upper case letters denote differences between exposure times.
Figure 7.3: Mean serum cortisol (mean ± S.E.M.) in gilts bled every 15 minutes for 4 hours before initial boar exposure (before), 1 hour immediately post boar introduction (during) and 4 hours following boar introduction (after) or transportation to a new pen only (controls).

Combined average category is the group mean of before, during and after values. Overall means represent average cortisol concentration at each exposure time. Responders showed estrus prior to 200 days of age while non-responders failed to show estrus prior to 200 days of age. Lower case letters denote differences between groups while upper case letters denote differences between exposure times.
exposed gilts that did not show estrus had higher ($P < 0.05$) cortisol levels than either the control gilts or boar exposed gilts showing estrus before 200 days of age (Figure 7.3).

7.3.5. Effect of boar exposure on estradiol

No significant difference in estradiol values was detected between any of the groups of gilts at any of the four times of exposure (Table 7.2). Additionally, due to very high variances, there was no significant age effect on estradiol concentrations (Table 7.2).

7.4. Discussion

Control gilts exhibited their first estrus at an age commonly reported for gilts not exposed to a boar (Kirkwood and Hughes, 1980a; Paterson and Lindsay, 1980; Kirkwood et al., 1981; Caton et al., 1986; Van Lunen and Aherne, 1987). Also, the age at first estrus in the responding gilts was similar to that published elsewhere (Kirkwood and Hughes, 1979; Paterson and Lindsay, 1980; Eastham et al., 1984; Eastham and Cole, 1987) and to the average for the herd from which our gilts were purchased (B. Andries, pers. comm.).

The effect of boar introduction and exposure on LH secretion in the present study paled in comparison to that seen with the ram effect. When a ram is introduced to anestrous ewes, the increase in LH pulse frequency is
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dramatic (Martin et al., 1983). The only group of gilts that showed significant changes in LH secretion following boar exposure was the group which eventually exhibited signs of first estrus. Unlike ewes, however, in gilts the increased LH secretion was transient and no long term trend, indicative of a cascade leading to first estrus and ovulation, was seen. In prepubertal lambs and gilts, the onset of first estrus is preceded by an increase in LH pulse frequency (Rawlings and Churchill, 1990). However, the limited changes in LH secretion seen in gilts in response to a boar in this study could have been sufficient to trigger some maturational mechanism resulting in early first estrus.

Mean and basal LH levels also increased transiently in the boar exposed gilts which showed estrus. This was most likely due to the increase in LH pulse frequency since the change in the mean LH concentration occurred in the same time frame.

There appeared to be no acute response of serum concentrations of FSH to boar introduction. FSH concentrations fell to values lower than those reported elsewhere (Colenbrander et al., 1982; Diekman et al., 1983; Camous et al., 1985, but depressed FSH secretion would not appear to be a reasonable explanation for boar induced onset of first estrus in the gilt as low FSH concentrations would interfere with follicular development and delay the onset of puberty.
Serum concentrations of estradiol did not follow the trends in either LH or FSH secretion. No significant changes in serum concentrations of estradiol were seen, but over the period of boar exposure, serum concentrations of estradiol appeared to be elevated and quite variable in gilts that did not show estrus before 200 days of age. There was no evidence for gonadotrophic stimulus for these changes in serum concentrations of estradiol. These observations indicate, as Foxcroft et al. (1984) have suggested, that a stable ovarian steroid environment is required for a normal response to the boar, in terms of early induced estrus.

Mean serum cortisol values generated in our study were similar to those published elsewhere (Nyberg et al., 1988). As expected, cortisol levels decreased throughout the day, as a result of normal circadian rhythms (Whipp et al., 1970). There was no transient increase in cortisol levels immediately after boar introduction or transportation as reported before, (Pearce and Hughes, 1987; Dalin et al., 1988) although pigs in this study were allowed only pen-line and not full physical contact, in contrast to the study of Pearce and Hughes (1987). When serum concentrations of cortisol were pooled for the whole day of boar introduction, it was seen that cortisol levels were higher in boar exposed gilts that did not show estrus by 200 days of age, compared to the boar exposed gilts which did show estrus or controls gilts (Table 7.3). If cortisol is taken as an indicator of stress, it would appear that the boar exposed gilts not cycling before 200 days of age
were more stressed than the others and that this extra stress could have interfered with the boar's induction of estrus. This is in contrast to previous speculation that an increase in cortisol, mediated through the stress of relocation and mixing with new pigs, is an essential component of boar induced estrus (Pearce and Hughes, 1987). Our study suggested that those gilts which released the most cortisol were those which were unlikely to show signs of early estrus. This corroborated a study by Dalin et al. (1988), in which it was concluded that high levels of cortisol following transport stress inhibited the early onset of estrus in gilts.

It has been postulated that the mechanism of boar induced estrus involves the male stimulation of a release in cortisol, which in turn causes an increase in basal LH levels (Fujihara and Shiino, 1980; Liptrap and Raeside, 1983). This elevated basal LH may result in accelerated follicular development and estradiol production leading to the first pre-ovulatory surge. Again, however, our results indicated that increased cortisol, following pen-line contact for estrus stimulation, was inhibitory on the onset of estrus cycles in prepubertal gilts. Additionally, there was no transient increase in serum concentrations of cortisol with boar introduction, but LH pulse frequency increased for a short time in boar exposed gilts that experienced an early estrus. This suggested that the boar's signal was not a cortisol message to the hypothalamic-pituitary axis or else the cortisol signal was inhibitory, and not stimulatory, in the gilts not showing estrus in
response to the boar. In a more recent study (Pearce and Paterson, 1992), it was also concluded that cortisol was not a mediator of boar induced estrus in the prepubertal gilt.

7.5. Conclusions

The ability of the boar to induce estrus in gilts does not involve an increase in serum concentrations of cortisol. Cortisol does not appear to be a mediator of increased LH pulse frequency at boar introduction. In fact, cortisol may be inhibitory to the ability of the boar to induce first estrus in the gilt. The hormonal milieu following boar introduction to gilts that did not respond by showing estrus was characterized by low fluctuating FSH, high and variable estradiol concentrations and stable LH pulse frequencies. In contrast, gilts responding with estrus showed a transient increase in LH pulse frequency, a low and stable serum concentration of estradiol and a decrease in serum FSH concentration. No single major endocrine change would appear to be a candidate for the early induction of first estrus in the gilt following boar exposure. This is in contrast to the effect of the ram on anestrus ewes, where a rapid and marked increase in LH pulse frequency precedes ovulation (Martin et al., 1983). In the gilt however, a transient increase in LH pulse frequency following boar introduction, may be sufficient to mediate boar induced estrus.
7.6. Future research proposals

Further research could involve the collection of blood samples every 15 minutes for variable times after initial boar exposure to determine the length of the transient increase in LH pulse frequency.

Pulsatile administration of exogenous LH at high frequencies over various time frames, in the absence of a mature boar could be followed by analysis of the number of gilts which exhibited, and failed to exhibit, first estrus. This could further elucidate the exact role of increased LH pulse frequency in the gilt.

Gilts could also have their endogenous LH pulse frequencies augmented through exogenous administration of LH immediately following initial boar exposure. The null hypothesis would state that increased LH pulse frequency would have no effect on the percentage of gilts exhibiting first estrus in response to boar exposure.
8.0. Effect of administration of exogenous LH on age at first estrus and on serum LH and FSH

8.1 Introduction

The percentage of gilts failing to exhibit estrus by 200 days of age is a major source of economic loss in the commercial swine industry. Many studies have attempted to determine the cause of this failure to exhibit normal estrus cycles or conversely to determine management and drug therapy programs to increase the percentage of cycling gilts.

Drug therapy has mainly centred around the use of GnRH, PMSG and hCG combinations and, more recently, estradiol benzoate. However most of the therapies have produced undesirable side effects such as failure to exhibit normal estrous cyclicity (Lutz et al., 1985), lower than normal embryonic survival rates (Paterson et al., 1984), abnormal cyclicity with asynchronous ovulation (Kirkwood and Aherne, 1988) and decreased ovulation rates and cystic follicles (Dyck, 1988). Additionally, the practicality of such treatment is questionable, since delayed puberty is genetically determined and offspring from such gilts are poor candidates for replacement gilts.

Increases in LH pulse amplitude, pulse frequency and mean serum concentrations between 70 and 135 days of age suggest that this may be an important priming phase for future sexual maturation. This, coupled with
results from Chapter 5 showing that mean serum LH concentrations at 80 days of age may allow differentiation between future cycling and non-cycling gilts, led to the postulation that administration of exogenous LH at 80 days could have an impact on the percentage of gilts eventually exhibiting first estrus before 200 days of age. It was hypothesized that some of the gilts which would not otherwise have exhibited first estrus, due to low mean LH concentrations at 80 days of age, may exhibit first estrus before 200 days of age if endogenous LH concentrations were augmented around 80 days of age.

8.2. Materials and Methods

8.2.1. Animals

Two groups of ten weaner gilts (Y x L), 61 ± 2 days old, were purchased from the PSRC and moved to the IHU in the WCVM. The two replicates were started 10 days apart. All gilts were initially housed in concrete-sided pens (3.1m X 4.6m, 12L/12D) in groups of 10. At 85 days of age, gilts were grouped 5 per pen in the same sized pens.
8.2.2. Experimental Protocol

Ten days following transport to the WCVM all gilts were ear-tagged and randomly assigned to either the control (saline injected, N=5 per replicate) or treatment (LH injected, N=5 per replicate) groups.

When gilts were 75 days of age, LH (NOBL Laboratories, Sioux Centre, Iowa; 15 mg porcine LH = 25 units of NIH porcine LH standard; dose = 50 µg/0.2 ml I.M. per injection) was injected I.M. every 6 hours for 10 days. Control gilts received an equivalent volume of saline. Gilts were herded into a tight packed group during injections but were not physically restrained with a snout snare.

At 78 days of age, gilts were catheterized and placed in individual carts (0.75 m X 1.5 m) for 48 hours. Twelve ml of saline was flushed through the catheters 4 times daily. Carted gilts were bucket fed twice daily and were given fresh water every 4 hours. At 80 days of age, 5 ml blood samples were drawn every 15 minutes for 8 hours for LH and FSH analysis. LH injections continued during the intensive sampling period. Catheters were removed at the end of the 8 hour period.

At 135 days of age, gilts were exposed to mature males and checked daily for external signs of estrus (see General Materials and Methods). Gilts which exhibited external signs of estrus only once, and did not commence regular estrous cycles, were designated as non-cycling gilts.
8.2.3 Radioimmunoassays

Intra- (n=4) and inter- (n=8) assay coefficients of variation for LH were 2.3 % and 23% respectively for a reference serum (mean = 1.8 ng ml\(^{-1}\)) replicated in each assay. Serum from both replicates was analyzed for FSH in a single assay. The intraassay (n=13) coefficient of variation for FSH was 5.6% for a low reference serum (mean = 7.9 ng ml\(^{-1}\)) and 7.0% for a high reference serum (mean = 12.9 ng ml\(^{-1}\)).

8.3.3 Statistical Analysis

Replicate was included as an independent variable in a model statement for two-way repeated-measures analysis of variance (SAS Version 6, Statistical Analytical Systems, Cary, North Carolina) and was not found to have a significant effect on the data (P < 0.11). Thus, both replicates were combined into one data set for statistical analysis.

LH pulse amplitude data determined from intensive blood samples from 80 day old gilts, was log transformed to correct heterogeneity of variance (P < 0.03). LH frequency, mean and basal concentration data were homogeneous (P < 0.73, P < 0.90, P < 0.66 respectively) as were mean serum FSH concentration data (P > 0.9), so no transformations were required.

Comparisons of LH pulse amplitude, LH pulse frequency, mean LH and basal LH concentrations, serum concentrations of FSH, and age at first
estrus, between control and treatment groups were obtained using an unpaired two-tailed T-test (SAS Version 6) with a 95% confidence interval. Frequencies of cycling and non-cycling gilts in the control and treatment groups were compared by Chi-square analysis (SAS Version 6).

8.3. Effect of exogenous LH administration on age at first estrus and on circulating serum LH and FSH concentrations.

8.3.1. Effect of exogenous LH on age at first estrus

Mean age at first estrus for control gilts was not significantly different from that of treatment gilts (154.2 ± 3.4 vs. 148.9 ± 1.9 days respectively, P < 0.42).

Within the control group, 60% of gilts showed regular estrous cycles by 200 days of age and 40% of gilts failed to exhibit estrus by 200 days of age, whereas within the LH treatment group 80% of gilts exhibited estrus and only 20% of the gilts failed to exhibit regular estrous cycles by 200 days of age. Chi-square analysis of these data resulted in a chi-square value of 0.952 and a P value of 0.32.
8.3.2. Effect of exogenous LH on circulating serum LH and FSH concentrations

Basal serum concentrations of LH were significantly (P < 0.004) elevated in treatment gilts compared to controls gilts but serum LH pulse amplitude, pulse frequency and mean concentrations were similar between the two groups (Figure 8.1).

Mean serum concentrations of FSH were also similar between control and treatment gilts (7.6 ± 0.4 vs. 7.7 ± 0.4 respectively, P < 0.7).
Figure 8.1: Luteinizing hormone pulse amplitude (ng ml\(^{-1}\)), pulse frequency (pulses hr\(^{-1}\)) and mean (ng ml\(^{-1}\)) and basal (ng ml\(^{-1}\)) LH concentrations (all values are means ± S.E.M.) in gilts treated with saline (control) and gilts treated with 50 μg of exogenous LH (treatment) 4 times daily between 75 and 85 days of age. Lower case letters denote differences between groups.
8.4 Discussion

Results from the prediction study described in Chapter 4 indicated that gilts with mean LH levels greater than 1 ng ml\(^{-1}\) of serum at 80 days of age were more likely to exhibit first estrus before 200 days of age than were gilts with LH values less than 0.75 ng ml\(^{-1}\) at the same age. In this experiment, 10 gilts were given 200 μg of porcine LH daily between 75 and 85 days of age. It was postulated that elevation of circulating serum LH concentrations over a ten day period would alter the LH and FSH hormone profiles of gilts and influence, either positively or negatively, the percentage of gilts exhibiting their first estrus.

The preliminary nature of this investigation did not warrant the completion of a dose response curve to determine which dose of LH was the most effective at eliciting a response. Instead, the dose of exogenous LH utilized was derived from a previous study in which either 68μg or 1 mg of LH was administered to 200 day old gilts (Esbenshade and Day, 1981). Both doses were ineffective at increasing the number of gilts exhibiting first estrus when compared to a control group. In our study, a daily dose of 200μg was administered to 80 day old gilts, a dose 4 times greater than the lowest used by Esbenshade and Day (1981).

Although age at first estrus was no different between control and treatment gilts, the percentage of gilts exhibiting first estrus in the treatment group was double that seen in the control group. This suggested
that treatment with exogenous LH was successful in stimulating estrus in some gilts which otherwise would have failed to exhibit normal estrous cycles.

The basal serum LH concentration seen in the treatment group was higher than that seen in the control group. This confirms that administration of exogenous LH was effective in altering one aspect of the LH hormone profile when the gilts were 80 days of age. However, LH pulse amplitude, frequency and mean concentrations were not affected. In 80 day old gilts pulsatile LH release, LH pulse amplitude, LH pulse frequency and mean and basal serum concentrations are increased compared to values seen in gilts younger or older that 80 days. (Lutz et al., 1983; Camous et al., 1985; Pelletier et al., 1987). In addition, in 80 days old gilts, the uterus and associated physical structures are growing and the ovaries are producing their first crop of tertiary follicles in response to LH levels which are much higher than those seen in adult cycling gilts (Lutz et al., 1984). In this study, LH pulse amplitude and frequency as well as mean serum LH concentrations were similar to those published elsewhere in gilts of similar ages (Colenbrander et al., 1977; Camous et al., 1985; Ponzilius et al., 1986) and only the basal serum LH concentrations of the treatment gilts were higher than previously published data (Colenbrander et al., 1977). This lends support to the hypothesis that it is elevated basal serum LH concentrations that are responsible for the increased percentage of gilts
exhibiting first estrus in our treatment group. It could also be suggested that it was a high basal serum LH concentration that was responsible for the high mean serum LH concentrations in 80 day old gilts that eventually showed estrus before 200 days of age as discussed in Chapter 4. Further, high basal serum LH concentrations at the time of tertiary follicle development could be an essential precursor of the gilts' ability to respond to boar exposure by exhibiting first estrus before 200 days of age.

Esbenshade and Day (1981) concluded that single doses of exogenous LH given to 200 day old gilts on the day of relocation had no effect on the incidence of first estrus. Our studies suggested that a better age range for the administration of LH was between 75 and 85 days of age. A single dose of LH would be insufficient since the result would be a transient perturbation of the LH profile and not a sustained elevation of basal serum LH concentrations as seen in our study.

Unlike basal serum LH concentrations, mean serum FSH concentrations for both the control and treatment gilts were very similar to each other and to those published previously (Camous et al. 1985). We hypothesized that, if administration of exogenous LH altered the serum LH profile, there could be a concomitant change in the feedback pattern to the pituitary and the hypothalamus. Since some gonadotropic cells of the pituitary are responsible for both LH and FSH secretion, it was further postulated that changes in the serum LH profile might alter the release of
FSH thereby contributing to an alteration of the percentage of gilts exhibiting first estrus. However, no change in mean serum FSH concentrations was detected between the two groups in this study.

Two gilts had circulating mean serum FSH concentrations much lower (1.7 ng ml\(^{-1}\) and 1.3 ng ml\(^{-1}\)) than the other gilts in this study. However, one gilt began cycling at 138 days of age and the other failed to exhibit signs of estrus before 200 days of age. Therefore no relationship between mean serum FSH concentrations and future reproductive status could be inferred.

8.5. Conclusions

Administration of 200 \(\mu\)g of exogenous LH per day was insufficient to produce a significant elevation in the percentage of gilts exhibiting first estrus. It was, however, sufficient to double basal serum LH concentrations. Further research is necessary before the effect of the treatment is known.

8.6. Future research proposals

Replication of this study will require a large increase in the number of experimental animals in order to produce an accurate Chi-Square analysis comparing the frequency of gilts showing and not showing estrus between treatment and control groups.
A dose response study is necessary to determine the dose which will elevate mean serum LH concentrations would then allow a different approach to investigate the relationship between increased serum LH concentrations and age and first estrus.

The minimum and maximum necessary time frames for elevation of basal LH concentrations that will produce the increased incidence of first estrus will require further experimentation. This study utilized a ten day period surrounding 80 days of age. In Chapter 5, it was shown that, at 80 days of age, the mean serum concentrations of LH were different in gilts which subsequently exhibited estrus by 200 days of age when compared to those which failed to exhibit estrus by 200 days of age. It is possible that a decreased number of injections over fewer days, or an increased dose of exogenous LH over fewer days, would produce the same result. Also, to reduce labour, automatic sub-cutaneous pumps could be inserted and removed at the appropriate ages with a minimum of contraindications for the gilt.
9.0 General Discussion

Hypothesis #1: That, in gilts less than 120 days old, the concentrations of LH, FSH and estradiol, individually or in combination, correlate with eventual age at first estrus.

The ability to accurately identify gilts with a poor reproductive future would generate some savings for commercial swine producers. A test to identify these gilts must be cost efficient, quick and have widespread applicability. To begin to develop any such test it is first necessary to determine if a relationship exists between age at first estrus and the mean serum concentrations of either LH, FSH or estradiol.

In this study, it was determined that one blood sample taken at 81, and analyzed for mean serum LH concentration, yielded data that was representative of age at first estrus such that segregation of potentially non-cycling summer gilts appeared possible. However, this relationship between age at first estrus and mean serum LH concentrations was not found in gilts which reached the same age but in the winter season. This indicated that season was an important external variable in the determination of the relationship. Further experimentation is needed to determine if the different seasonal results are due to changes in photoperiod, temperature of a combination of both.
The difficulty encountered during this study with respect to seasonal differences in the serum LH patterns would give any commercial LH/first estrus detection test too narrow a season of applicability. Any test that is limited in scope is of questionable market value. Producers are unlikely to participate in widescale testing if the results are accurate for only one part of their production year. Data from the blood test at 81 days of age, however, showed a difference in the hormone patterns of gilts which eventually exhibited regular estrous cycles or failed to exhibit regular estrous cycles. This difference has the potential of being exploited for further scientific research and possibly, commercial gain.

Of the three hormones analyzed, only mean serum LH concentrations were related to age at first estrus. Mean serum FSH and estradiol concentrations were not correlated, individually or in combination with LH values, with age at first estrus in gilts. Thus it appeared that, at 81 days of age, it is maturation of the LH secretory process, that is critical for normal sexual maturation. As the process of sexual maturation is complicated and involves many physical systems, it seems unusual that a changes in a single hormone secretion profile can have such a profound effect on the age at first estrus in the gilt. However, increases in mean serum LH concentrations have been shown to stimulate the final steps to the first ovulation in sheep (Martin et al., 1978; Poindron et al., 1980) so a similar mechanism in the gilt may not be novel.
Further research is required to determine whether the relationship between age at first estrus and mean serum LH concentrations detected in our summer gilts will be of some use in herds of different genetic and breed backgrounds, and, whether the relationship, used over several generations, can significantly improve the quality of gilts entering into the replacement herd. The ideal scenario would be selection of and breeding for gilts which naturally exhibit first estrus before market weight. Those failing to do so would be culled at market weight with no ensuing overweight penalties for the producer.

This issue of cost and savings is of utmost concern to the producer. In a commercial 250-sow herd the average annual budget will often exceed $350,000 (Saskatchewan Pork Producers Board, 1990 Statistics). A future commercial test to identify gilts with good and poor reproductive capabilities will likely cost approximately $15.00 per gilt (all figures are 1991 figures). In a 250-sow herd, with 88 replacement gilts and 22 extra gilts required annually, a commercial method based on the LH/age at first estrus relationship data, if generalized to all seasons, would cost $1650. Since the producer is estimated to incur approximately $2400 in stockyard penalties per year, the total savings amount to $750.

Seven hundred and fifty dollars represents only 0.2% of the annual budget of an average 250-sow commercial operation. While this may seem insignificant the intangible benefits are, by far, the most lucrative. Any
such future test based on the LH/age at first estrus relationship has the potential of enabling a producer to increase the genetic potential of the herd. Isolation and removal of gilts with less than ideal reproductive futures leaves only gilts with promising reproductive futures in the replacement herd. Over only a few generations this genetic selection will greatly improve the genetic quality of the herd. It remains to be determined if the selection of gilts which will attain early estrus will have any long term effect on fecundity, litter size, litter number or ability to maintain condition.

Use of a test to identify gilts which will fail to exhibit normal estrous cycles before 200 days of age will be useful for basic scientific research. In both the boar exposure (see Chapter 7) and the hormone therapy (see Chapter 8) studies, the ability to accurately pre-select gilts which are likely to fail to exhibit normal estrous cycles would enable the reliable inclusion of adequate numbers of gilts in the treatment groups. In the boar exposure study, further research could include intensive investigation of the LH pulse frequency patterns in non-responding gilts early in sexual development and then following boar exposure. Identification of non-responding gilts could also be followed by a determination of the effectiveness of a combination of exogenous LH therapy and administration of high frequency LH pulses following boar exposure. If treatment such as this is effective at stimulating gilts to exhibit estrus, the identification of some of the hormonal
some of the hormonal shortcomings in non-responding gilts would be more complete and available for extensive research. However, the use of such a test results in a scenario for which it is difficult to provide authenticity. Identification of gilts with a poor future reproductive performance, coupled with hormone therapy and boar exposure to stimulate normal first estrus can never be verified. It can never be known for certain that the identified gilts would definitely have had a poor reproductive future. Thus, to be useful scientifically, any test developed from the relationship between mean serum LH concentrations and age at first estrus must be widely verified under many management systems, in many breed and breed-combinations, in many geographical location and under many different ambient temperature and lighting regimes.

Therefore, while the data generated by this study forms a potential basis for more promising research, more extensive experimentation is needed before there is any commercial or scientific application.
Hypothesis 2: That changes in LH or FSH secretion in gilts less than 120 days old reflects a critical endocrine maturational change essential for the normal expression of first estrus before 200 days of age.

Results generated from the prediction study suggested that, in 81 day old gilts which failed to exhibit estrus before 200 days of age, overall pituitary LH secretion was altered when compared to LH secretion in gilts which did exhibit estrus before 200 days of age. However, attempts to investigate which aspect or aspects of the LH secretion profile were responsible for this difference in absolute serum LH concentrations were unsuccessful (see Chapter 6). Difficulties involving the repeated cannulation of very young animals appeared to interfere with hormone secretion such that the hormone patterns observed in Chapter 5 gilts were not found in Chapter 6 gilts. More consistent results could likely have been obtained had a long-term, non-stressful method of blood collection been available.

The different lighting regimes in operation in each of the Chapters 5 and 6 experiments also represent a confounding variable. The Chapter 5 gilts were housed in a dark barn with minimal ambient or incandescent light while gilts in the Chapter 6 study were housed under fluorescent lights timed to produce 12 hours each, of light and dark. While the effects of photoperiod can be said to be equivocal (Ntunde et al., 1979; Awotwi and
Anderson, 1985) on age at first estrus in the gilt, it is not known what specific effect the different photoperiods may have on mean serum LH secretion or what general effect they may have on the hypothalamo-hypophyseal-gonadal axis in the gilt.

Research for the study conducted in Chapter 6 was completed, as it was for replicates #1 and #3 in Chapter 5, in the summer, albeit two years later. The mean ambient temperatures in degrees Celsius, as well as the extreme monthly temperatures, for each of the two years (1988 and 1990 respectively) were as follows (from Environment Canada):

**Table 9.1:** Monthly average and extreme temperatures for June, July, August and September in 1988 and 1990 (Environment Canada).

<table>
<thead>
<tr>
<th>YEAR</th>
<th>MONTH</th>
<th>JUNE</th>
<th>JULY</th>
<th>AUGUST</th>
<th>SEPTEMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>Average</td>
<td>21.2</td>
<td>19.2</td>
<td>16.5</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>Extreme</td>
<td>41</td>
<td>37</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td>1990</td>
<td>Average</td>
<td>17.1</td>
<td>17.7</td>
<td>18.0</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>Extreme</td>
<td>32</td>
<td>31</td>
<td>35</td>
<td>32</td>
</tr>
</tbody>
</table>

Little difference can be seen between the average ambient temperatures throughout the summer in each year but there was a marked difference, between 1988 and 1990, in the extreme temperatures in both June and July. It appears, however, that the hormone profiles in Chapter 5 gilts were not affected by the high temperatures. Indeed, there was no statistical difference between the hormone patterns in Chapter 5, replicate #1 gilts sampled in 1988 and the replicate #3 gilts sampled in 1990. Chapter 6 gilts
were raised under the same conditions as Chapter 5 gilts until the former were moved to the WCVM at 28 or 34 days of age. Once in the WCVM, the gilts were subjected to climate control and thus were removed from wide fluctuations in ambient temperature. Why the Chapter 6 gilts, sampled in 1990 should present such different hormone profiles than did the Chapter 5 gilts must therefore be attributed to factors other than ambient temperature, such as photoperiod, cannulation stress or changes from ambient temperature to climate control. Therefore, to achieve better results in a study such as that completed in Chapter 6, more careful control of the environmental conditions to which the gilts are subject, is required to eliminate several possible sources of inconsistency.

The increase of LH pulse frequency in gilts which respond to boar exposure with first estrus (see Chapter 7) suggested that inadequate LH pulse frequency could be responsible for abnormal sexual development. Conversely, exogenous LH therapy at 81 days of age resulted in increased basal LH concentrations (see Chapter 8). While this increase in basal serum LH levels did not significantly lower age at first estrus in the treatment gilts, the increased percentage of gilts in the treatment group which exhibited first estrus indicated that increased serum basal LH may also be a critical part of the maturational changes associated with the expression of normal estrus.
Again, use of the relationship between mean serum LH concentrations and age at first estrus (see Chapter 5) would be useful as it would ensure sufficient numbers of non-responding gilts for comparison to responding and control gilts.

Therefore, the data generated from the LH/age at estrus study (Chapter 5) suggested that a critical maturational process occurs in the prepubertal gilt at about 81 days of age. This is supported by changes in mean serum LH concentrations in gilts which respond to boar exposure with first estrus before 200 days of age and the lack of change in serum LH concentrations in gilts which failed to respond to boar exposure with first estrus before 200 days of age.

Objective 3: That gilts which respond to boar exposure by exhibiting estrus before 200 days of age have a different hormone profile than do gilts which fail to respond to exposure and that these differences represent the endocrine mechanism of boar induced first estrus.

The results of the LH/age at first estrus study (see Chapter 5) indicated that 81 day old gilts which eventually failed to exhibit first estrus before 200 days of age were deficient in mean serum LH concentrations. Gilts which failed to respond to boar exposure with first estrus did not show the same post-boar exposure increase in LH pulse frequency that was seen
in gilts which did exhibit first estrus in response to boar exposure (see Chapter 7). Further, in gilts treated with exogenous LH at 81 days of age, basal serum LH concentrations were elevated, as was the percentage of gilts exhibiting first estrus, compared to control gilts (see Chapter 8). All of these results indicate that it is maturation of all facets of the pituitary LH secretory system which is critical for the normal time of expression of first estrus.

In 135 day old gilts which failed to exhibit first estrus, the impact of environmental stimuli, mediated through increased levels of cortisol compared to control gilts or gilts which exhibit estrus, appear to be involved in the interference of normal sexual development.

Therefore gilts which fail to exhibit first estrus in response to boar exposure do have different hormone profile than gilts which do respond to boar exposure. This difference likely begins around 80 days of age when maturation of the LH secretion mechanism is abnormal and continues when the gilts are unresponsive to the presence of a boar. Environmental and possibly physical stress also appears to have an impact on final maturation occurring around the time of boar introduction.
Conclusions

Maturation of the reproductive tract requires physical development in synchrony with appropriate hormonal changes. Significant ovarian development occurs between 60 and 90 days of age with the appearance of tertiary follicles (Oxender et al., 1979). At the same time, in normal gilts, mean serum LH concentrations are increasing (Pelletier et al., 1981), overall ovarian and uterine weights are increasing (Dyck and Swierstra, 1983) and steroid negative feedback is evident (Elsaesser, 1982). In this study, we determined that gilts which fail to exhibit first estrus before 200 days of age, under normal management conditions, begin to show deviations in their hormone patterns, when compared to gilts which do exhibit first estrus before 200 days of age, as early as 81 days of age. Gilts 135 days of age, which fail to exhibit estrus before 200 days of age, also show a marked difference in their ability to respond to the presence of a boar when compared to normal gilts. This suggests that if some, or all, of the pituitary maturation is not completed before puberty, introduction of a boar is insufficient to stimulate the maturation at a later age and the gilt will fail to exhibit normal estrous cyclicity.

We have demonstrated that a key component of the boar's effect on the gilt appears to be a stimulation of LH pulse frequency. Ovulation does not follow as quickly after introduction of a boar as it does with the ram
effect on the ewe (Riches and Watson, 1954; Watson and Radford, 1960). However, from the initial stimulation of puberty, to stimulation of estrus in the post-weaning sow, the boar’s effect on female pigs is a significant component of modern swine management. Knowledge that LH pulse frequencies are elevated following exposure to a boar will now allow further research into the mechanism of the boar effect.

Hormone therapy at 81 days of age was postulated to have an effect on the percentage of gilts exhibiting estrus before 200 days of age. Again, interfering with hormone patterns at this age appeared to have an effect on normal sexual maturation in the gilt since a somewhat higher percentage of gilts in the treatment group exhibited estrus compared to the percentage of gilts in estrus in the control group. This further corroborates the conclusion that a critical time for sexual maturation occurs early in development.

At 81 days of age in the gilt, it appears necessary that, not only must the ovaries grow and develop LH hormone receptors, but that the pituitary and hypothalamus must mature at the same time and produce an LH secretory profile much more dynamic than before 81 days of age. At 135 days of age, the reproductive tract of the gilt must be ready to respond to boar exposure with an increase in LH pulse frequency in order for final sexual maturation to occur. The culmination of proper pre-pubertal development of the gilt’s LH secretory system and astute management is normal estrous cyclicity before 200 days of age.
The existence of a relationship between mean serum LH concentrations and age at first estrus in 81 day old summer gilts suggests that further research will provide insight into the two broadest questions addressed by this study—why do some gilts fail to exhibit normal estrus before 200 days of age and why do some gilts fail to respond to boar exposure with first estrus?
10.0. References


