CONTROL OF OVULATION IN THE PIG USING A GnRH ANTAGONIST

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USING A GnRH ANTAGONIST

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This thesis is dedicated to my Dad,

Cecil B. Patterson.

I love you, Daddy!

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CHAPTER I

INTRODUCTION

Reproductive efficiency (# piglets/sow/year) is one of the most important economic factors in commercial swine production. Nutritional and environmental management, combined with early weaning practices, may increase the number of pigs that a sow will rear in a year. However, the largest reduction in the potential number of piglets born to each sow occurs during early gestation and is caused by internal factors for which a manager has little or no control. Pigs generally ovulate 14 to 16 ova, but only 9 to 10 of these will be represented by piglets born alive and only 7 to 8 of these survive to weaning (Pope and First, 1985). The loss of embryos during early gestation is referred to as embryonic mortality and is defined as the difference between the number of corpora lutea (CL) present on the ovaries and the number of live embryos present before day 30 of gestation. Embryonic loss averages 30% prior to day 40 of gestation followed by an additional loss of 10-20% from day 40 of gestation until term (Flint et al., 1982; Pope and First, 1985). Relatively little information is available regarding the factors which cause embryonic mortality during the early stages of gestation.

Early embryonic mortality may be due to variation in the time of follicle ovulation relative to the onset of estrus and the stage of follicular development at ovulation (for review see Hunter et al. 1993a). Pope et al. (1988) reported that ovulation occurs in a majority/minority pattern, that is, the majority of follicles ovulate in a short period of time followed by the minority ovulating over a more protracted interval. The group of follicles that constitute the "minority" are thought to be less developed both functionally and morphologically than those that constitute the "majority." Destruction of the lesser-developed "minority" of follicles by electrocautery resulted in a more uniform embryo population on day 11 of gestation (Pope et al., 1988).

Geisert et al. (1982b) reported that a rapid change in morphology occurs in early embryos from day 11 to 12 of gestation. The early conceptus diameter expands to approximately 10 mm through cellular hyperplasia by day 11 to 12 of gestation, then rapidly elongates within 2 to 3 hours to a thin filamentous form. This transformation occurs through cellular remodeling with expansion of the trophoblast throughout the uterine horn at a rate of 30-45 mm/hr, reaching up to 150 mm in length upon completion. Therefore, small variations in the time of ovulation and fertilization may lead to large variations in conceptus morphology on days 11 to 12 of gestation. Also, Geisert et

al. (1982a) found that the conceptus initiates production of estrogen coincident with its rapid elongation. The more developed conceptuses that were possibly ovulated from the majority follicles and fertilized earlier may start production of estrogen before the lesser developed embryos are capable of surviving in the estrogenic environment. Studies have demonstrated that administration of estradiol before days 11 to 12 of gestation resulted in embryonic death (Pope et al., 1986; Morgan et al., 1987; Pope and First, 1985; Gries et al., 1989).

Blair et al. (1994) and Hunter et al. (1993b) reported that embryo survival increased as the time from onset of estrus to peak of plasma luteinizing hormone (LH) concentration and follicle ovulation increased. These data suggest that embryos arising from follicles ovulating later in the estrus period have a better chance for survival during the subsequent period of rapid elongation and initiation of estrogen production. It is possible that a later time of ovulation relative to onset of estrus allows the "majority" subset of follicles to grow and be synchronized in their maturity at time of ovulation.

The present thesis was developed to determine a protocol to regulate the time of the LH surge and subsequent ovulation in the pig in order to ascertain the affects that timing of the LH surge relative to the onset of estrus have on embryo survival. Experiments were

designed to establish the effective dosage of a Gonadotropin Releasing Hormone (GnRH) antagonist which would inhibit LH secretion during the preovulatory surge and block ovulation in the gilt. If such a strategy were successful it could be combined with the use of human chorionic gonadotropin to control the exact time of ovulation relative to onset of estrus in the pig.

The following review of the literature provides the background information on endocrinology of the estrous cycle, events surrounding ovulation, and factors affecting early embryonic mortality in swine. At this time no information has been published to describe the use of GnRH antagonists in the pig. However, there are numerous papers which provide information on GnRH antagonists in other domestic farm species.

CHAPTER II

REVIEW OF LITERATURE

Porcine Estrous Cycle

Estrous Cycle

The length of the pig estrous cycle ranges from 19 to 23 days with the average interestrus interval being approximately 21 days. Duration of sexual receptivity or behavioral estrus is approximately 30 to 48 hours for gilts which is slightly shorter than that of 30 to 72 hours for sows (Anderson, 1993). Ovulation usually occurs between 36 to 40 hours after the initial onset of estrus (Hunter, 1994) and has been reported to require approximately 4 hours to complete (Anderson, 1993). However, there is considerable variation in the time of ovulation. It can occur before, during or several hours after the first detection of standing heat.

The estrous cycle of domestic farm species can be divided into two phases: the follicular phase and the luteal phase. The follicular phase, which is the period of follicular growth in the absence of corpus luteum (CL), consists of proestrus and estrus while the luteal phase, a period of dominance by the CL, consists of metestrus and

diestrus. Endocrine profiles of the hormones involved with the estrous cycle of the pig are represented in Figure 1.

Follicle Stimulating Hormone

During proestrus, concentration of estrogen in plasma rises while the concentration of follicle stimulating hormone (FSH) declines in an apparent inverse relationship. This inverse relationship is evidenced by an FSH surge at the time of estrus, coinciding with the rapid decline of plasma estradiol-17β (E_2) at the time of ovulation (Van De Wiel et al., 1981). During the FSH surge, the maximum plasma concentration of FSH ranges from 11.3 to 18.5 ng/ml (Van De Wiel et al., 1981) and occurs approximately 1 to 5 hours after the peak concentration of LH. A secondary rise in plasma FSH occurs on day 2 to 3 of the estrous cycle (first day of estrus = Day 0). The peak concentrations of FSH during the second surge approximates 20 ng/ml and this surge coincides with low plasma E_2 concentration following ovulation. During the remainder of the estrous cycle, the plasma concentration of FSH remains at approximately 7 ng/ml. (Rayford et al., 1974).

Estradiol-17β

Concentration of estradiol-17β in plasma begins to increase between days 16 to 18 of the estrous cycle, peaking at approximately 38 pg/ml approximately 2 days prior to initiation of estrus (Guthrie et al., 1972). The increase in plasma estrogen is coincident with declining plasma progesterone concentration and final maturation of the Graafian follicles. Plasma estrogen concentration steadily declines to approximately 25 pg/ml at estrus and remains low (< 20 pg/ml) throughout the luteal phase of the estrous cycle (Hansel and Echternkamp, 1972).

Luteinizing Hormone

The high plasma concentration of estrogen produced by the Graafian preovulatory follicles prior to estrus stimulates the surge release of luteinizing hormone (LH) which results in subsequent ovulation in the absence of elevated progesterone from the CL. The LH surge results from the sustained plasma concentration of estrogen which forms a positive feedback loop on the hypothalamus and anterior pituitary (Van De Wiel et al., 1981). Surge release of LH into the peripheral vascular system from the anterior pituitary occurs about 40-42 hours prior to follicle ovulation (Liptrap and Raeside, 1966) and reaches

peak plasma concentrations of 4-6 ng/ml approximately 12 hours after the initiation of the surge release (Parvizi et al., 1976; Van De Wiel, et al., 1981). Plasma concentration of LH returns quickly to basal levels (< 1 ng/ml) following the ovulatory surge (Van De Wiel et al., 1981) for the remainder of the estrous cycle.

Progesterone

Plasma progesterone (P_4) is low (< 0.5 ng/ml) during estrus and starts to increase after ovulation on day 2 of the estrous cycle. Progesterone reaches peak plasma concentrations ranging from 33 to 35 ng/ml on days 12 to 14 of the estrous cycle (Guthrie et al., 1972; Hansel and Echternkamp, 1972). If the animal is not pregnant, a rapid decline in P_4 occurs on day 15 reaching basal levels by day 17 to 18 (Guthrie et al., 1972). The decline in plasma P_4 concentration is due to the regression of the functional CL caused by a release of prostaglandin $F_{2\alpha}$ from the non-pregnant uterine endometrium on days 12 to 14 of the estrous cycle (Moeljono et al. 1976; Bazer et al. 1982). Regression of the CL by $PGF_{2\alpha}$ and subsequent decrease in P_4 concentration restarts the cycle over again, unless interrupted by pregnancy or some type of endocrine dysfunction.

Ovulation

Follicular Growth Patterns

Follicular growth in the pig is initiated during the luteal phase of the estrous cycle as evidenced by the increase in the cohort of small follicles. Each ovary of the gilt contains a pool of approximately 50 small follicles ranging from 2 - 5 mm in diameter during the late luteal and early follicular phase of the estrous cycle (Anderson, 1993). Follicles that are destined for ovulation increase to 8 - 11 mm between days 14 to 16 of the estrous cycle (Anderson, 1993). Experiments involving electrocautery of follicles (Clark et al., 1979) and unilateral ovariectomy (Coleman and Daily, 1979; Clark et al., 1982) also demonstrated that recruitment of follicles for ovulation occurs between days 14 to 16 of the estrous cycle. Grant et al. (1989) collected ovaries from naturally cycling pigs on days 16, 18, 20, and 21 of the estrous cycle and found a progressive decrease in the number of follicles per animal. These data support the suggestion by Clark et al. (1973) that there is a "physiological block" that prevents replacement of atretic follicles in the proliferating pool during the follicular phase.

The decrease in follicle number was accompanied by an increase in the average size of the follicles that were present, with almost all follicles present on the ovaries measuring greater than 8 mm in diameter on day 21. Grant et al. (1989) also investigated other characteristics of follicle maturation, including follicle diameter, follicular fluid volume, follicular concentrations of estradiol, testosterone and progesterone, and granulosa cell number in all follicles greater than or equal to 2 mm. Additionally, they measured granulosa cell aromatase activity and theca testosterone content by ^{125}I-labeled hCG binding to granulosa and theca cells. Their results showed a strong positive relationship between follicle diameter and volume on all days. The number of granulosa cells recovered per follicle varied but was not related to estrogenic activity of the follicles. Mean follicular fluid estradiol and testosterone concentrations and ^{125}I-labeled hCG binding increased until day 20 and then decreased on day 21, whereas mean theca testosterone content, ^{125}I-labeled hCG binding to theca tissue and aromatase content were all at maximum levels on day 21.

On days 20 and 21, a subset of approximately 14-16 large ($\geq$ 7 mm) follicles were distinguishable from the remaining smaller follicles. However, within the group of large follicles, there was a difference of approximately 2 mm in follicular diameter as well as considerable variation

in the biochemical development of follicles of the same size. In a similar study by Hunter et al. (1989), in which follicles were recovered just before ovulation, many of the follicles had become flaccid and the follicular fluid had an increase in viscosity suggesting that ovulation was imminent. However, there was considerable variation in follicular diameter and steroid concentrations of the follicular fluid of the pre-ovulatory follicles. Hunter et al. (1990) stated that the results of these two studies demonstrated that morphological and biochemical variation between follicles exists up to the immediate preovulatory period. Variation in follicle morphological and biochemical development in the peri-ovulatory period may be related to differences in the time of ovulation and/or embryo quality within a litter.

Timing of the LH Surge

The pre-ovulatory LH surge occurs about 40-42 hr before ovulation (Liptrap and Raeside, 1966) and attains peak levels of 4-6 ng/ml approximately 12 hours after the initiation of the surge (Parvizi et al., 1976; Van De Wiel et al., 1981). Soede et al. (1994) reported similar results in that the interval from peak LH concentration to ovulation varied from 26 to 34 hours with a mean of 30 hours. It is not clear, however, when the LH surge occurs

relative to the onset of standing estrus. Niswender et al. (1970), Henricks et al. (1972) and Parvizi et al. (1976) reported that the LH surge occurred during the onset of estrus. Findlay et al. (1974) and Aherne et al. (1976) reported that the preovulatory surge of LH occurred from 24 to 36 hours before estrus in weaned sows. Ziecik et al. (1982) found that the preovulatory LH surge was not coincident with the onset of estrus in sows. Ziecik et al. (1982) using sows in the postweaning interval, found that the preovulatory LH surge occurred 8 to 32 hr before detection of standing estrus in (7 sows) or after the onset of standing estrus (7 sows).

In a recent study by Blair et al. (1994), the time from the onset of estrus to peak LH concentration varied among pigs categorized as high embryo survival and low embryo survival females. The LH peak occurred 11 hr after onset of standing estrus in high embryo survival females but 5 hr after onset of estrus in sows categorized as low embryo survival. The investigators stated that the timing of the LH peak relative to the onset of estrus appears to be involved in determining the duration of estrus. Soede et al. (1995) stated that the moment of ovulation is strongly related to the duration of estrus. These researchers indicated that ovulation occurred after approximately 70% of the gilt's estrus period was complete instead of ovulation occurring at a constant interval of

time (36-40 hours) after the onset of estrus (Hunter, 1994). Thus, the time of ovulation varies relative to the length of estrus expression in each individual female. However, the time interval from the peak of the LH surge to ovulation remains at a fixed time of approximately 30 hr regardless of the length of estrus expression.

Synchrony of Ovulation

The pattern of ovulation in the pig has been a difficult biological event to investigate. Depending on the experimental technique used, the duration of the ovulatory process varies considerably. Anderson (1993) stated that the ovulatory process takes approximately 3.8 hours. Pope et al. (1988) indicated that the duration of ovulation occurred in a majority/minority pattern, with 68-95% of follicles ovulating in a short period of time, while the remaining minority ovulated over a more protracted interval. Pope et al. (1988) used 113 crossbred gilts in 3 different experiments to examine the relationship between the pattern or sequence of ovulation and subsequent developmental variation in day 11 embryos. The percentage of follicles that had ovulated at 26, 30, 34, or 38 hours after the onset of estrus and at 39, 41, 43, 45, or 47 hours after injection of human chorionic gonadotropin was determined using mid-ventral laparotomy in the first

experiment. The percentage of follicles that had ovulated in 52 gilts at a set time of 34 hours after the onset of estrus was evaluated using mid-ventral laparotomy in experiment 2. In experiment 3, sham operations and electrocautery of non-ovulated follicles was done on day 1 of estrus in gilts (n=8/group) to evaluate variation among littermate embryos on day 11 of gestation. The results of these experiments indicated that the initiation of ovulation in naturally ovulating gilts occurs from 30 to 34 hours after the onset of estrus and from 39 to 41 hours after injection of hCG in gilts artificially induced to ovulate. The pattern of ovulations appeared to be skewed with 68 - 95 % of follicles ovulating in a short period of time and the remainder of the ovulations taking a longer time. In the third experiment, destruction of the smaller number of later ovulating follicles resulted in a more uniform group of day 11 embryos with fewer small embryos.

Attempts have been made to come up with a less invasive method to study the ovulatory process. Soede et al. (1992) conducted a study to determine if transrectal ultrasonography could be used to effectively study the process of ovulation. They used transrectal ultrasonography every thirty minutes from 5 to 20 hours before the onset of ovulation to determine maximum follicle count on both ovaries. The number of follicles observed with ultrasonography only differed from actual corpora

lutea count at slaughter by 0.4 ± 1.8. In the spontaneously ovulating sows in their study, the duration of ovulation was 1.8 ± 0.6 hours and in sows induced to ovulate with human chorionic gonadotropin ovulation lasted for 4.6 ± 1.7 hours.

Early Embryonic Loss

Early Embryonic Loss

The majority of embryonic loss in the pig occurs during the early stages (d 10-30) of pregnancy. Embryonic mortality ranges from 20-40% from days 10 to 30 of pregnancy with an average loss of 30% (see Flint et al., 1982; Pope and First, 1985 for review). The majority of this early embryonic loss occurs before day 25 of gestation which corresponds to the time period when the conceptus is undergoing rapid trophoblastic elongation, uterine migration, placental attachment and maternal recognition of pregnancy (see Geisert et al. 1990).

Many different theories have been proposed to explain the causes of embryonic mortality. The majority of early embryonic mortality does not result from fertilization failure, chromosomal abnormalities, insufficient luteal development or insufficient uterine space (Pope and First, 1985). An early theory of Bazer et al. (1969) proposed

that embryos compete for some unknown biochemical substance produced by the uterus that is in limited supply during early development. Competition for this unknown substance might be influenced by differences in morphological development of embryos within a litter. Anderson (1978) observed that considerable variation in embryonic morphology exists within a litter as early as day 12 of gestation. Embryos within a given litter ranged in shape and size from spherical (0.1 to 4 mm) to filamentous (approximately 150 mm in length) forms.

Pope et al. (1986) examined the importance of uniform embryo development by utilizing asynchronous superinduction and evaluated conceptus survival and development on days 12 to 13 of gestation and embryo survival to day 30 of gestation. These researchers transferred day 6 embryos into day 7 pregnant recipients and day 7 embryos into day 6 pregnant recipients. Upon examination of the embryos on day 12 or 13 of gestation, they found morphological variation from spherical to filamentous conceptuses that was within the limits of what had been reported previously for naturally mated sows. Conceptuses recovered from the day 6 embryos transferred into day 7 pregnant recipients were morphologically more variable and proportionately less developed when compared to day 7 embryos transferred into day 6 pregnant recipients. The researchers also found that more day 7 embryos transferred into day 6 pregnant

recipients survived to day 30 of gestation than day 6 embryos into day 7 pregnant recipients. Thus, conceptuses which are advanced in development will compete more successfully for survival than those earlier stage embryos.

Such variation in the naturally occurring population of early embryos within a litter may be due to variation in duration of ovulation and/or development of follicles at the time of ovulation. As previously discussed, Pope et al. (1988) investigated the relationship between pattern of ovulation and morphological variation among embryos within a litter on day 11 of gestation. Electrocautery of all nonovulated large Graafian follicles at 34 hours after onset of estrus resulted in a more uniform embryo population on day 11 of gestation when compared with sham cauterized controls. These data indicated that the later ovulating follicles most likely develop into the small conceptuses and that the earlier ovulating follicles become the more developed conceptuses present on day 11 of gestation.

Variation in morphological development due to time of ovulation may be important for embryo survival during the period of rapid trophoblastic expansion that occurs from day 11 to 12 of gestation. Geisert et al. (1982b) described the morphological changes that take place in early embryos as they transform from a spherical to filamentous form. Those researchers found that following

embryo hatching from the zona pellucida on day 8, the conceptus increases in diameter at a rate of 0.25 mm/hour, reaching 10 mm by day 11 to 12 of gestation. Shortly after reaching the 10 mm spherical stage, the conceptus elongates to a filamentous form at a rate of 30-45 mm/hour reaching up to 150 mm within 2-3 hours. Geisert et al. (1982b) also demonstrated that the early growth to the 10 mm spherical stage involves cellular hyperplasia. Whereas, the rapid elongation to the filamentous form of the embryo occurs through cellular remodeling and not cellular hyperplasia.

Geisert et al. (1982a) found that the embryo initiates production of estrogen during the time of rapid trophoblastic elongation on days 11 to 12 of gestation. Estrogen produced by the developing conceptus has been implicated as the signal for maternal recognition of pregnancy in swine (see Bazer et al., 1982, 1986; Geisert et al., 1990). However, the very estrogen that signals the maternal system to maintain CL function may be detrimental to the health of embryos that were formed from oocytes of follicles that ovulated later and therefore, were less developed at the time of conceptus elongation.

Administration of estradiol before days 11-12 of gestation resulted in embryonic death (Pope et al., 1986; Morgan et al., 1987; Pope and First, 1985; Gries et al., 1989). Pope et al. (1986) found that administration of 2.0 mg of estradiol-17β on days 9 and 10 of gestation caused

the death of embryos in a majority of gilts while doses of 8.0 mg/day or greater resulted in complete embryo loss in all gilts by day 30 of gestation. Morgan et al. (1987) administered estrogen before the conceptus normally releases estrogen to determine the affect of a premature estrogen signal on development of the conceptus and endometrium. These researchers treated pregnant gilts with estradiol valerate on day 9 or days 9 and 10 of gestation. They found that conceptuses underwent normal trophoblastic elongation and appeared normal on day 11 and 12 of gestation. However, by day 16, the conceptuses were fragmenting and degenerating. Pope and First (1985) also found that administration of estradiol-17β on days 9 and 10 resulted in embryo death in a majority of sows when assessed on day 30, whereas, treatment with the same dosage on days 12 and 13 of gestation had no detrimental affects on embryo survival. Thus, estrogen administration causes loss of embryos when given prior to normal conceptus steroid production but has no affect during the time the conceptus normally secretes estrogen thereby ruling out a direct toxic affect of exogenous estrogen on the pig embryos. Gries et al. (1989) treated gilts with 5 mg of estradiol valerate and found that embryos elongated normally through days 9 and 10 of gestation but they failed to survive past day 14. Blair et al. (1991) indicated that estrogen affects embryonic survival through an alteration

of endometrial function. Administration of estradiol valerate on days 9 and 10 of gestation resulted in a loss of the uterine glycocalyx beginning on day 14, which was coincident with the onset of embryonic mortality in the estrogen-treated gilts. These researchers concluded that the maternal glycocalyx was involved with placental attachment.

Studies in Meishan Pigs

Chinese Meishan pigs have high embryo survival and a large litter size. Studies in these prolific Chinese Meishan pigs have provided information regarding ovarian and endocrine function relative to the periovulatory period. Meishan pigs have a larger litter size and higher early embryonic survivability when compared with Large-White gilts. Comparative studies of the number of live births in Meishan compared with European white breeds found that the average litter size of 13-17 piglets for Meishan gilts was on average 3 to 4 pigs per litter greater than the European breeds (Haley et al. 1993). Studies comparing ovulation rate between Meishan and European breeds have reported varying results (see Haley et al. 1993 for review). Part of this discrepancy may be due to whether the comparisons were made on animals of the same age or on animals that had experienced the same number of estrous

cycles after puberty. However, when sows of higher parity are compared, there seems to be no breed difference in ovulation rate (Haley et al., 1993). Further studies are needed to accurately determine if there is any variation in ovulation rate between the two breeds.

Haley et al. (1993) stated that the key to the prolificacy of Meishan pigs was enhanced prenatal survival. The difference in prenatal survival between the two breeds is approximately 15% to term, with most of this difference occurring by day 20 of gestation and possibly earlier. Hunter et al. (1993) reviewed the endocrine and follicular differences between the Meishan and European white breeds. They reported that the overall patterns of estradiol, LH and FSH secretion were not different between the Meishan and Large-White gilts. Behavioral estrus was exhibited earlier relative to the LH surge in Meishan gilts (49 hours from onset until LH surge) than in Large-White hybrid gilts (34 hours from onset until LH surge). However, the time interval from the peak of plasma estradiol concentration until the peak of the LH surge was not different between the two breeds. These data suggest that the Meishan is more sensitive to estradiol in relation to initiating a behavioral response, but not in relation to positive feedback to move up the timing of the LH surge relative to the onset of standing estrus.

Preovulatory follicular characteristics in the Meishan gilts were found to be as variable as in the Large-White, but follicles from Meishan gilts were smaller (6.5 versus 7.2 mm) and contained a higher concentration of estradiol (319 versus 243 ng/ml) in follicular fluid than Large-White gilts. The oocyte population in the Meishan gilts was also found to be at a more advanced stage of development preceding ovulation than in the Large-White. Hunter et al. (1993) also reported that decreasing the interval from the onset of estrus to ovulation by advancing the LH surge in the Meishan using hCG injections decreased embryo survival at day 30 of gestation. Therefore, they concluded both endocrine and follicular mechanisms play a role in the prolificacy of the Meishan breed.

Causes of Embryo Mortality

Ford and Young (1993) disagree with the conclusion of Pope et al. (1990) that littermate variation is the major cause of embryo mortality and suggest that research should focus on conceptus-uterine interactions as the major cause of embryonic mortality. Ford and Young (1993) present data from their laboratory to show that preimplantation Meishan embryos develop more slowly and produce less estrogen than embryos of the less prolific European breeds but still express diversity within the litter. They suggest that

there is a factor(s) within the endometrial secretions of Meishan pigs that reduce growth rate and estrogen secretion of preimplantation embryos. Therefore, lower estrogen production by the Meishan embryos in a Meishan uterus may result in a more gradual change in the uterine environment that is beneficial for embryo survival and litter size.

Variation in the time of ovulation may lead to morphological diversity within a litter during day 8 to 10 when the synthesis of estrogen is initiated. Variation in conceptus development may play an important role in early embryonic mortality. This is supported by the reports of Blair et al. (1994) and Hunter et al. (1993) that show increased embryo survival as the time from onset of estrus to peak LH and ovulation increased. These data suggest that if tight regulation for initiation of the LH surge and ovulation could be achieved, possibly through the use of GnRH antagonists, a practical method to synchronize ovulation and increase embryo survival in the European breeds could be developed.

GnRH Antagonists

GnRH Antagonists

One possible way to control the LH surge and ovulation may be through the use of GnRH antagonists. Lincoln and

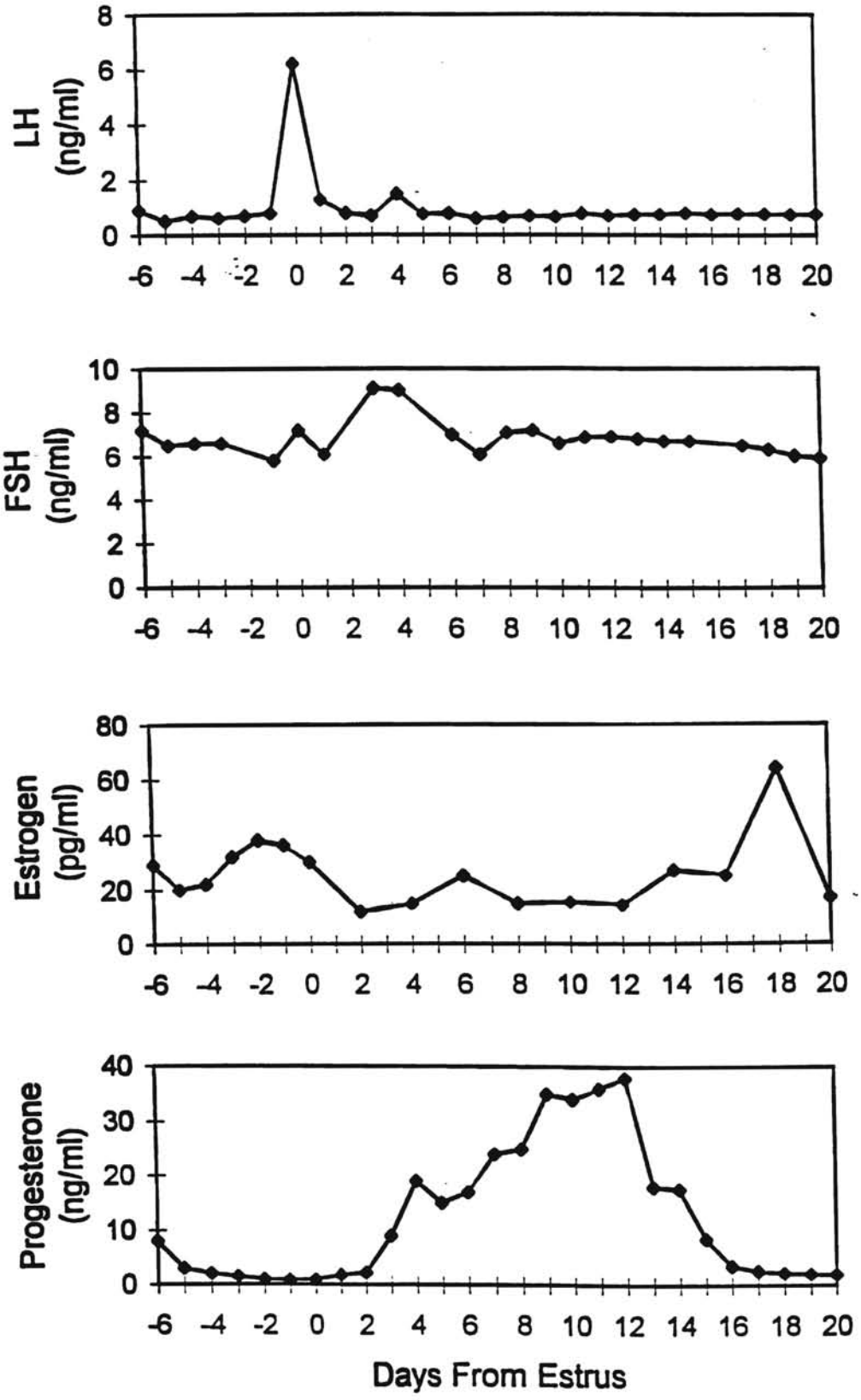
Fraser (1987) found that iv infusion of a LHRH antagonist (RS-18286, Syntex, Palo Alto, CA, USA) immediately blocked LH pulse secretion in sexually active rams. These researchers found that the duration of LH inhibition was dose dependant. The low dose of 6 μg/kg blocked LH pulsatility for 4.3 ± 0.4 h, the medium dose of 36 μg/kg inhibited LH secretion for 18.0 ± 1.0 h, and the high dose of 365 μg/kg inhibited LH pulses for 31.8 ± 1.3 h. In a pilot study, Campbell et al. (1990) reported that a single s.c. injection of 50 μg/kg of a GnRH antagonist (Contraceptive Development Branch, Center for Population Research, National Institute for Child Health and Human Development, NIH, Bethesda, MD, USA), completely inhibited endogenous pulsatile LH secretion for at least 24 hr during the early follicular phase of ewes. McNeilly et al. (1992) treated ewes with 1 mg. kg^{-1} BW of a GnRH antagonist (Org. 30276, batch J, Organon, Oss, Holland) on day 4 or day 11 of the estrous cycle and found that the antagonist abolished pulsatile secretion and suppressed basal LH concentrations for at least 3 days after treatment. Estrus was delayed until at least day 22 in animals that were treated with the GnRH antagonist on day 11 of their estrous cycle.

Similar results have been reported in cattle. Rieger et al. (1989) administered 0.4 or 0.8 mg/head of a GnRH antagonist (synthesized by D.H. Coy, Tulane University

School of Medicine) intravenously at the first sign of behavioral estrus and again 12 hours later. Both doses significantly delayed the time of ovulation as determined by transrectal ultrasonography in Holstein heifers. The mean time from onset of estrus to ovulation in the 1.6 mg dose group was 74 hours compared with approximately 20 hours in the control group. In a second experiment, the investigators found that a total dose of 1.5 mg of GnRH antagonist given in split injections at 48 and 60 hours after cloprostenol injection significantly delayed the growth of the ovulating follicle, onset of estrus, the preovulatory LH surge and ovulation as compared to control animals. In another study, Madill et al. (1994) used the same GnRH antagonist (synthesized by D.H. Coy, Tulane University School of Medicine) in superovulated Holstein heifers and found similar results. The researchers reported a dose-dependent decrease in the amplitude of the LH surge and delays in the time and occurrence of the LH surge and ovulation of the follicles. The time from the first injection of prostaglandin to the peak in the LH surge was 41.0 $\pm$ 1.3 hours in the controls compared with 70.6 $\pm$ 1.2 hours in the heifers treated with the highest dose of antagonist (8.0 mg). The LH surge in the controls reached a concentration of 29.8 $\pm$ 3.7 ng/ml, whereas the 8.0 mg treated heifers only reached 4.1 $\pm$ 1.3 ng/ml.

The aforementioned studies indicate that GnRH antagonists may be utilized to successfully delay the onset of the LH surge and ovulation in ruminants while allowing follicular growth to occur. Therefore, the objective of the present research was to determine if a GnRH antagonist could be effectively utilized to block the LH surge and subsequent ovulation in gilts. If this objective is accomplished, it will provide a useful model to study the affect of regulating the LH surge and the time of ovulation relative to the onset of estrus and its subsequent affects on embryo survival in the pig.

Figure 1. Profiles of plasma concentrations of progesterone, estrogen, follicle stimulating hormone and luteinizing hormone during the estrous cycle in the pig (Adapted from Anderson, 1993).


LH (ng/ml)
8
6
4
2
0
-6 -4 -2 0 2 4 6 8 10 12 14 16 18 20
FSH (ng/ml)
10
8
6
4
2
0
-6 -4 -2 0 2 4 6 8 10 12 14 16 18 20
Estrogen (pg/ml)
80
60
40
20
0
-6 -4 -2 0 2 4 6 8 10 12 14 16 18 20
Progesterone (ng/ml)
40
30
20
10
0
-6 -4 -2 0 2 4 6 8 10 12 14 16 18 20
Days From Estrus

CHAPTER III

CONTROL OF LUTEINIZING HORMONE SECRETION AND OVULATION IN THE GILT USING A GnRH ANTAGONIST

Introduction

Early embryonic mortality is one of the major factors contributing to reproductive inefficiency in commercial swine production. Death rate of embryos averages approximately 30% before day 40 of gestation with an additional loss of somewhere between 10-20% from day 40 until term (Pope and First, 1985). One of the possible causative factors involved with the loss of embryos before day 30 of gestation may be diversity in development of the early conceptus population within a litter (Pope et al., 1988; Xie et al., 1990). Numerous studies have indicated that variation in spherical diameter and development exists from day 10 to 12 of gestation in pigs (Anderson, 1978; Geisert et al., 1982; Stroband, 1992). The variation of development among conceptuses has been suggested as a possible cause for at least a portion of the early loss of some embryos.

Conceptus variation in morphological and biochemical development during the early period of gestation may be

related to the range in time of ovulation or maturation stage of individual follicles at ovulation. Previous studies by Pope et al. (1988) indicated that porcine follicles ovulate in a "majority/minority" pattern. The majority of follicles ovulate in a very short period, whereas the remaining minority ovulate over a more protracted interval. Removal of the later ovulating "minority" of follicles by electrocautery resulted in a more uniform population of embryos on day 11 of gestation.

Asynchronous superinduction studies by Pope et al. (1986) demonstrated that transfer of embryos that were 24 hours younger or older than embryos within the pregnant recipient created an increased morphological variation on days 12 to 13 of gestation. Evaluation of embryo survival on day 30 of gestation indicated that more advanced (older) embryos had greater competitive survivability compared with embryos 24 h behind in development. Geisert et al. (1982 a,b) demonstrated that the events involved with rapid trophoblastic elongation and initiation of estrogen production by the porcine conceptus occurs between day 11 to 12 of gestation. The more developed conceptuses that were, possibly from follicles that were ovulated and the eggs subsequently fertilized earlier, may have a competitive advantage over the later ovulating follicles that result in the lesser developed conceptuses as they are the first to quickly establish their placental space within

the uterine horns (see Pope et al. 1990). Estrogen production by these more developed conceptuses also starts before the lesser developed conceptuses are capable of surviving in an estrogenic environment. Studies have shown that administration of estradiol before days 11-12 of gestation result in embryonic death (Pope et al., 1986; Morgan et al., 1987; Pope and First, 1985; Gries et al., 1989).

Time of ovulation is controlled by initiation of the LH surge, however there is considerable variation in when the LH surge occurs relative to the initiation of estrus behavior. The surge in serum LH may occur before, during or after first detection of behavioral estrus (Niswender et al., 1970; Henricks et al., 1972; Parvizi et al., 1976; Findlay et al.. 1974; Aherne et al., 1976; Ziecik et al., 1982) Data from Blair et al. (1994) and Hunter et al. (1993b) indicate that embryo survival increased as the interval from onset of estrus to time of the LH surge and ovulation increased. The increase in embryo survival may be a result of allowing all developing Graafian follicles destined to ovulate, more of a chance to catch up developmentally and attain a synchronous stage of maturation before ovulation. Thus, it is possible that an LH surge during estrus establishes a more synchronized ovulation and oocyte maturation and results in a better chance of conceptus survival during the period of rapid

trophoblast elongation and initiation of estrogen production.

The aforementioned studies suggest that, if time of the LH surge and ovulation could be regulated, a method might be developed that would increase embryo survival during early gestation. One paradigm to control the time of ovulation may be through the use of GnRH antagonists to inhibit the ovulatory surge of LH. Studies by Lincoln and Fraser, (1987), Campbell et al. (1990) and McNeilly et al. (1992) in sheep and Rieger et al. (1989) and Madill et al. (1994) in cattle indicate that GnRH antagonists may be utilized to successfully inhibit secretion of LH and delay ovulation. At present, we have no knowledge of any information published on the effectiveness of GnRH antagonists in the pig. Therefore, the objectives of the present study were to first, establish an effective dose of a GnRH antagonist which would inhibit LH secretion in the pig and second, to inhibit the luteinizing hormone surge and ensuing ovulation in gilts. Utilization of a GnRH antagonist to inhibit the LH surge will provide a model to study regulation of the LH surge and time of ovulation relative to the onset of estrus and its subsequent effects on embryo survival in the pig.

Materials and Methods

Experiment 1

Mixed breed barrows (n=3) of approximately the same age (6 to 8 months) and weight (89 - 103 kg) were used to determine an effective dosage of a GnRH antagonist Ac-D-p-Cl-Phe^{1,2}, D-Trp^{3}, D-Arg^{6}, D-Ala^{10} GnRH (Org. 30276, batch J; kindly supplied by Dr. H.J. Kloosterboer, N.V. Organon, Oss, Holland) to inhibit basal secretion of LH. Barrows were fitted with indwelling jugular catheters after induction of anesthesia with a 5% solution of thiopentone sodium (Abbott Laboratories, Chicago, IL, USA) and maintained on a closed circuit system of halothane (2-5% Fluothane: Aveco Co. Inc., Fort Dodge, IA, USA) and oxygen (1.0 liter/minute). Tygon Microbore tubing (Norton Performance Plastics, Akron, OH, USA) was inserted into the jugular vein and exteriorized to the back of the neck with a trocar for ease of repeated blood sampling. After recovery from surgery, animals were randomly selected to receive a low (10 µg/kg BW), medium (50 µg/kg BW) or high (250 µg/kg BW) dose of the GnRH antagonist. GnRH antagonist was prepared in 10 ml of sterile saline less than 1 hour before intramuscular injection. Blood samples were collected at 30 and 15 minutes pre-treatment and at time 0, followed by every 30 minutes for the first 4 hours and then every 4 hours for a total of 48 hours post-

injection. Samples were allowed to clot overnight at 4°C and then centrifuged at 2500 RPM for 15 minutes at 4°C. Serum was harvested and stored at -20°C until radioimmunoassay for serum LH concentration.

Experiment 2

Mixed breed gilts (n=9) of approximately the same age (13 to 15 months), weight (127 to 180 kg) and genetic background were checked for estrus twice daily (0730 and 1730 hours) with intact boars. The onset of behavioral estrus was considered to be day 0 of the estrous cycle. Onset of behavioral estrus was defined as the first time that the gilt would stand to be mounted by a boar. After experiencing at least one complete estrous cycle, gilts were randomly assigned to either a control group or one of two GnRH antagonist treated groups. On day 17 of the cycle all gilts were fitted with a jugular catheter as described in experiment 1 except that anesthesia was induced with an intramuscular injection of a mixture of 2.5 ml of Xylazine (Rompun®, Miles Inc., Agriculture Division, Animal Health Products, Shawnee Mission, KS, USA) and 2.5 ml of Ketamine HCl (Ketaset®, Aveco Co. Inc., Fort Dodge, IA, USA) mixed with Tiletamine HCl and Zolazepam HCl (Telazol®, Fort Dodge Laboratories, Inc., Fort Dodge, IA, USA) given at 0.01 to 0.025 ml per kg BW. After recovery from surgical catheterization, gilts were observed for estrus activity

every 4 hours. At the initiation of standing heat, control animals (n=4) were given an intramuscular injection of 10 ml of sterile saline and one group of GnRH antagonist treated animals (GnRH1) were injected i.m. with 100 μg/kg BW (n=2) or 200 μg/kg BW (n=1) of the GnRH antagonist. A second group (n=2) of GnRH antagonist treated gilts (GnRH2) were administered a dose of 200 μg/kg BW i.m. as soon as they showed interest in the boar, but prior to solid standing heat. The GnRH antagonist was prepared in 10 ml of sterile saline less than 1 hour before injection. Blood samples were collected at time 0, 0.5 h, and 1 h post-treatment, followed by every hour for the first 4 h and then every 6 h for approximately 60 h. Samples were handled as described in experiment 1 and serum was stored at -20°C until assayed for serum concentrations of LH and estradiol-17β (E_2). An additional blood sample was collected via venipuncture at mid-cycle (days 9 to 13) to determine serum progesterone (P_4) concentration.

Ovaries were evaluated for signs of ovulation approximately 30 to 33 h from initiation of estrus. The ovaries were exteriorized via mid-ventral laparotomy after induction of anesthesia as described by Schmitt et al (1993). Care was taken not to touch the follicles to minimize disturbance of the ovulatory process. If ovulation had not occurred by this time, a second surgical evaluation was conducted approximately 43 to 66 h from

initiation of estrus. All animals were treated with antibiotics (Penicillin G, The Butler Company, Columbus, OH, USA) post-surgery. Daily observations were conducted after the blood sampling ceased for behavioral estrus to determine the interestrus interval following treatment.

LH Radioimmunoassay

Serum samples were analyzed for LH using a double-antibody radioimmunoassay as previously described by Newton et al. (1987). Intra-assay and inter-assay coefficients of variation were 14.3% and 4.0%, respectively. Sensitivity of the assay was 0.24 ng. ml^{-1}.

Estradiol-17β Radioimmunoassay

Plasma concentrations of estradiol-17β were quantified by direct radioimmunoassay using double antibody reagents obtained from Diagnostic Products Corporation, Los Angeles, CA. Composition of the assay reagents and antibody dilutions were not included in the directions provided by the manufacturer. Assay standards were prepared in pig plasma that was treated previously with charcoal to remove endogenous steroids by absorption. Activated charcoal (Sigma Chemical Co., St. Louis, MO) was added to pig plasma and stirred at room temperature for 18-24 hr before the charcoal-plasma suspension (50 mg/ml) was sequentially centrifuged (4°C) at 3,000 X g for 30 min and then at

34,500 X g for 60 min. The plasma supernatant was recovered and filtered under vacuum 3 times through two clean Whatman #1 filters. Charcoal-absorbed plasmas (0.5 ml) were extracted with 5 ml diethyl ether (Mallinckrodt Chemical Inc., Paris, KY). The aqueous layer was frozen in a dry-ice acetone bath and the solvent supernatant collected and evaporated at 50°C under gentle nitrogen flow. The dried-down extract was reconstituted in 0.5 ml PBS buffer (pH 7.2, 0.1 M sodium phosphate, 0.15 M sodium chloride, 0.1% bovine serum albumin) and assayed using the same reagents as above but with estradiol standards prepared in PBS buffer. Only charcoal-absorbed plasma with an estrogen content not different from zero was used to prepare standards in the direct assay.

Estradiol-17β (Sigma) was dissolved initially in absolute ethanol and then in PBS to give a stock concentration of 4 ng/ml. Fifty microliters of 4 ng/ml were added to 0.95 ml charcoal-absorbed plasma to give the highest assay standard (200 pg/ml) which was then serially diluted in the charcoal-absorbed plasma to provide the remaining standards (3.125 - 100 pg/ml); the charcoal-absorbed plasma was the zero standard. Glass tubes (12 X 75 mm) received 0.1 ml antiserum solution and 0.2 ml standard or plasma unknowns. Tubes were vortexed and incubated overnight at 4°C before 0.075 ml of [^{125}I]estradiol-17β (approximately 12,000 cpm) were added,

tubes vortexed, and incubated for 2 hours at 4°C. Cold (4°C) precipitating solution containing second antibody and polyethylene glycol was added (1 ml), tubes vortexed and incubated for 30 min at 4°C before tubes were centrifuged (4°C) for 30 minutes at 3,000 X g. Tubes were decanted and the precipitated counts quantified in an automatic gamma counter (Micromedic Meplus).

The RIA was validated for quantifying plasma concentrations of estradiol-17β by determining assay parallelism, recovery, and precision. Serial dilutions of pig plasma that were diluted in charcoal-absorbed plasma inhibited binding of [^{125}I]estradiol-17β to antibody in a manner that paralleled inhibition by estradiol standards prepared in charcoal-absorbed plasma. Recovery of 12.5, 25, 50 and 100 pg estradiol-17β added (0.05 ml) to aliquots (0.95 ml) of untreated pig plasma (n = 4) was best described by the regression equation Y = 1.01X - 1.84 (r= 0.996), which are results indicating that the assay essentially measured 100% of the exact estradiol content in plasma. Intra-assay coefficient of variation (CV) ranged from 6 - 12% and inter-assay CV ranged from 10 - 15% for plasma pools containing 5, 35 and 105 pg/ml. Sensitivity of the assay averaged 1.5 pg/ml (0.3 pg/tube) when sensitivity was defined as the estradiol concentration equivalent to antibody-precipitated counts that were 2

standard deviations below the mean of counts in a series of zero-standard tubes.

Progesterone Radioimmunoassay

Plasma concentrations of progesterone were quantified by a direct, solid-phase radioimmunoassay kit according to the basic procedure provided by the manufacturer (Diagnostic Products Inc.). Serial dilutions of pig plasma diluted in zero standard inhibited binding of [^{125}I]progesterone to antibody in a manner that paralleled inhibition by progesterone standards. Recovery of 0.5, 1, 5, 10, 25 and 50 ng progesterone added (0.05 ml) to aliquots (0.95 ml) of pig plasma was best described by the regression equation $Y = 0.84X - 0.25$ ($r = 0.986$). Intra-assay and inter-assay coefficients of variation for bovine plasma pools containing 2.5 and 10.4 ng/ml progesterone were 5.5 and 6.2%, and 10.1 and 12.3%, respectively. Similar CV have been obtained for human and sheep plasma (personal communication, Dr. J.P. McCann, Clinical Hormone Laboratory, Oklahoma State University) and for canine and equine plasma pools (Reimers et al., 1991). It is assumed that CV for porcine plasma pools would be similar to the values for bovine, equine and canine plasmas. Sensitivity of the assay averaged 30 pg/ml (3 pg/tube).

Statistical Analyses

Serum LH and estradiol-17β concentrations were analyzed by split-plot analysis of variance using the General Linear Models procedure of SAS (Statistical Analysis Systems, 1990). The model included the whole plot effects of treatment and pig(treatment), and split-plot effects of time and the treatment*time interaction. The pig(treatment) effect was used as the error term to test treatment differences. Treatment differences in length of interestrus interval, mid-cycle serum progesterone concentration , serum estradiol 17β and LH concentrations were analyzed using the General Linear Models procedure (SAS, 1990) and a model that simply included the effects of treatment. Dunnett's t-test (SAS, 1990) was used to compare each treatment level to the control. A treatment effect on incidence of ovulation was tested by Chi-square analysis (Steel and Torrie, 1980).

Results

Experiment 1

There was a dose-dependent inhibition of LH secretion by the GnRH antagonist (Figure 2). Serum LH concentration was unaffected by the low dose (10 µg/kg BW) of GnRH antagonist (Figure 2A). However, the medium dose (50 µg/kg BW) and high dose (250 µg/kg BW) of GnRH antagonist decreased serum LH to basal levels within 30 minutes. Concentration of LH in serum following administration of the medium dose of GnRH antagonist remained at a nadir (<0.3 ng/ml) for approximately 12 h and returned to pre-treatment levels by 16 h post-injection (Figure 2B). Serum LH concentration after treatment with the high dose of GnRH antagonist remained at a nadir (<0.3 ng/ml) for the entire 48 h blood sampling period (Figure 2C). Based upon the results of this preliminary trial, the 100 µg/kg BW of GnRH antagonist was initially utilized to determine the effects of the GnRH antagonist on ovulation of gilts in experiment 2.

Experiment 2

Surgical evaluation of the ovaries of the control gilts indicated that gilts 52-2 and 14-2 ovulated by 30 and 33 h post-treatment and gilt 8-7 ovulated by the second surgery at 66 h (Table 1). The remaining control gilt 32-8 had not ovulated at her second surgery conducted at 43

hours. Further surgical evaluation of this gilt was not conducted to try to limit surgical trauma to the animal. However, evaluation of serum LH and mid-cycle progesterone (Table 1) suggested that the gilt ovulated after 60 h post-estrus. Evaluation of the ovaries in the two GnRH1 gilts (74-9 and 78-4) treated with 100 µg/kg BW of GnRH antagonist at the onset of estrus indicated that they had ovulated by 33 hours post-treatment. In order to determine if the 100 µg/kg BW dosage of the antagonist was sufficient to inhibit the ovulatory surge of LH, an additional gilt (18-7) was treated with 200 µg/kg BW of GnRH antagonist at the onset of estrus. However, this gilt had also ovulated at the first surgery at 33 hours post-injection.

Since the LH surge could be occurring prior to initiation of estrus behavior, GnRH antagonist was given prior to estrus in a second group of gilts. The second group of GnRH antagonist-treated gilts (GnRH2) were injected with 200 µg/kg BW of GnRH antagonist at first indication of interest in a boar, which turned out to be approximately 8 hours prior to the onset solid standing heat. Evaluation of the ovaries indicated that there were no signs of ovulation in one treated gilt (18-3) at second surgery performed at 53 h post-injection ($P<0.05$). However, a second gilt (2-1) had 10 unovulated and 3 ovulated follicles when evaluated during the second surgery at 57 h. Both of these gilts exhibited an extended period

of behavioral estrus for approximately 7 days in gilt 2-1 and 15 days in gilt 18-3. The interestrus interval in control and GnRH1 gilts was similar and ranged from 19 to 24 days (Table 1). However, the two GnRH2 gilts that were treated 8 hours prior to estrus expression exhibited an extended interestrus interval compared with control and GnRH1-treated gilts ($P<0.05$). Gilt 2-1 had an interestrus interval of 26 days and gilt 18-3 did not express behavioral estrus by the end of the study 27 days post-injection.

Mid-cycle plasma progesterone concentrations (Table 1) varied among treatment groups. Control gilts and GnRH1 treated gilts had elevated concentrations of progesterone ranging from 11.63 to 20.63 ng/ml. The GnRH2-treated gilt (18-3) which had not ovulated at the time of second surgery had a mid-cycle P_4 concentration (0.89 ng/ml) consistent with inhibition of ovulation. The second gilt in the GnRH2 treatment (2-1) had a sub-luteal concentration of P_4 (3.40 ng/ml) which reflects the partial inhibition of ovulation observed at the second surgery.

Radioimmunoassay of the serum samples indicated that the LH surge had occurred prior to onset of estrus in the two control gilts that ovulated by 30 to 33 h from initiation of estrus (Figure 3 C,D). However, a clear LH surge was evident after the onset of estrus in the two control gilts that ovulated at approximately 60 h (Figure 3

A,B). Similar results were also found in the GnRH1-treated animals. Gilts treated with GnRH antagonist at onset of estrus exhibited the surge in LH prior to onset of behavioral estrus (Figure 4 A,B,C). However, gilts treated with the GnRH antagonist when they first showed interest in a boar, approximately 8 hours prior to the onset of behavioral estrus (GnRH2) demonstrated no indication of an ovulatory surge in LH (Figure 5 A,B). Serum estradiol-17β concentrations in the two GnRH2 treated gilts (Figure 5) were greater ($P<0.05$) and were sustained throughout the sampling period when compared to the control (Figure 3) and the GnRH1 treated gilts (Figure 4). Estradiol-17β declined during estrus and before the LH surge of all control and GnRH1 gilts.

Discussion

Previous studies have demonstrated that GnRH antagonists will block the pulsatile secretion of LH in sheep. Lincoln and Fraser (1987) reported that treatment of sexually active rams with a LHRH antagonist inhibited pulsatile LH secretion. Campbell et al. (1990) and McNeilly et al. (1992) were also able to block pulsatile LH secretion in ewes for an extended period after treatment with a GnRH antagonist. Treatment of barrows with a GnRH antagonist in the present study inhibited pulsatile LH, which was dose dependent. The low dose (10 μg/kg BW) of GnRH antagonist did not affect basal LH secretion. However, the medium (50 μg/kg BW) and high dose (250 μg/kg BW) of GnRH antagonist effectively decreased LH secretion to its nadir within 1 hour. Duration of suppression also exhibited a dose-dependent response as the medium dose blocked basal LH secretion for 12 h post-treatment and the high dose inhibited pulsatile LH secretion for the entire 48 h blood sampling period.

GnRH antagonists have been utilized to delay the preovulatory LH surge and ensuing ovulation in cattle. Rieger et al. (1989) and Madill et al. (1994) indicated that treatment of Holstein heifers with a GnRH antagonist significantly delayed the time and occurrence of the LH surge and ovulation. In the present study, administration

of 200 μg/kg BW of GnRH antagonist 8 h prior to the first expression of estrus by the gilt blocked the LH surge and ovulation. Ovulation was inhibited totally in one gilt and partially in a second GnRH antagonist-treated female for at least 57 h following initiation of estrus. It would appear that LH release and ovulation were inhibited for an extended period of time as both gilts expressed estrus behavior for an extended period of time. Low, mid-cycle serum progesterone concentrations and high serum concentration of estradiol-17β during estrus also suggest that follicles were cystic. The one gilt which ovulated three follicles and had low serum progesterone concentration at mid-cycle returned to heat at 26 days post-treatment. However, the second treated gilt failed to exhibit a post-treatment estrus by the end of the experiment at 27 days.

Administration of GnRH antagonist (100 or 200 μg/kg BW) at the initiation of estrus (GnRH1) did not inhibit or delay in the present study. Serum LH and estradiol-17β concentrations did not differ from the controls. GnRH1 gilts expressed a normal mid-cycle serum progesterone concentration and interestrus interval compared with controls. Inability of the GnRH antagonist to effectively inhibit ovulation in the gilts treated with GnRH antagonist at the onset of standing estrus was not likely due to the

lower dosage of antagonist compared to GnRH2 gilts as 200 μg/kg BW was ineffective in one gilt as well.

Failure of GnRH antagonist in the GnRH1-treated gilts may have been due to the time of treatment relative to initiation of the LH surge. The profiles of serum LH and estradiol-17β indicated that the LH surge had occurred prior to our detection of estrus. Variation in the time of the LH surge relative to the onset of standing estrus was observed in the control gilts and has been reported in the literature. Niswender et al. (1970), Henricks et al. (1972) and Parvizi et al. (1976) reported that the LH surge takes place during the onset of estrus, whereas Findlay et al. (1974) and Aherne et al. (1976) reported that the LH surge occurred from 24 to 36 hours before estrus. Ziecik's et al. (1982) findings support the results of both groups in that the LH surge does not always coincide with the onset of estrus. Sows could have ovulatory surges of LH before standing estrus or after the onset of standing estrus (Ziecik et al., 1982). However, the time from the peak of the LH surge to ovulation appears to be approximately 30 h, no matter when the LH surge occurs relative to the onset of estrus (Soede et al., 1994). The present data indicate that the LH surge occurred before estrus in two control gilts which subsequently ovulated at approximately 30 hours from initiation of heat. Ovulation occurred at approximately 65 hours from detection of first

heat in the other two gilts in which the LH surge was clearly evident at approximately 35 hours from first expression of estrus.

The time of the LH surge relative to the onset of estrus can be directly linked to failure of the GnRH antagonist to inhibit ovulation in the three GnRH1 gilts. At the time of GnRH antagonist treatment in these gilts, serum LH concentrations were at peak concentrations or declining from peak values. We cannot clearly determine that the peak concentration of LH occurred before estrus since blood sampling was not initiated until heat detection. However initial serum samples in some gilts were elevated in LH, serum estradiol-17β was declining and ovulation occurred 30 h from initiation of estrus, suggesting that the LH surge had occurred. Therefore, the GnRH antagonist was not effective in gilts when the surge release of LH occurred before estrus.

Recent reports of Blair et al. (1994) and Hunter et al. (1993b) suggest that the later the time of the LH surge and ovulation relative to the onset of estrus, the higher the prolificacy of gilts and sows. Increased embryo survival reported by these researchers may be due to the later time of the LH surge allowing for establishment of a more complete oocyte maturation and synchronized ovulation of all follicles. Pope et al. (1988) have shown that variation in the time of ovulation and oocyte maturation

may lead to morphological diversity among embryos at day 12 of gestation. Those researchers indicated that electrocautery of later ovulating follicles leads to a more uniform embryo population on day 12 of gestation, suggesting that the later ovulating follicles become the lesser developed embryos that are present at that stage of gestation. Thus, it is possible that a later LH surge establishes a more synchronized ovulation and complete oocyte maturation of all follicles which results in a better chance of conceptus survival during the period of rapid trophoblast elongation and initiation of estrogen production (see Geisert et al., 1994).

The present study has demonstrated that a GnRH antagonist administered approximately 8 h before estrus expression will block the LH surge and ovulation. This study provides a model to determine the effect that inhibiting the endogenous LH surge and timing ovulation with human chorionic gonadotrophin relative to the onset of estrus has on subsequent embryo survival as proposed by Blair et al. (1994). Variation in time of occurrence of the LH surge relative to the onset of estrus can be overcome if the GnRH antagonist is administered 12 h prior to initiation of heat. Timing for administration of the GnRH antagonist can be regulated through synchrony of gilts with altrenogest or weaning of sows. Our laboratory is currently utilizing the GnRH antagonist to inhibit the LH

surge and treating females with human chorionic gonadotropin to stimulate ovulation at various time intervals relative to initiation of estrus. If timing of the LH surge relative to estrus affects embryonic survival and subsequent prolificacy in the pig, it will be possible to develop a protocol for utilization by the swine industry.

Table 1. Time of Ovulation, Number of Ovulated Follicles, Mid-Cycle Progesterone and Interestrus Interval of Gilts Treated with Saline or GnRH Antagonist

Treatment	Gilt Number	Time of[1] Ovulation (hours)	Number of[2] Ovulated Follicles	Progesterone[3] ng/ml	Interestrus[4] Interval (days)
Control					
	8-7	66	10	20.63	21
	52-2	30	12	11.63	19
	14-2	33	14	13.16	20
	32-8	>43	0(13)[5]	19.40	20
GnRH1					
	74-9	33	13	19.16	24
	18-7	33	10	18.15	19
	78-4	33	15		19
GnRH2					
	2-1	>57	3(10)[5]	3.40	26
	18-3	>53	0(13)[5]	0.89	>27

[1]Time of surgical evaluation of ovaries relative to the onset of treatment.

[2]Number of ovulated follicles present on the ovaries.

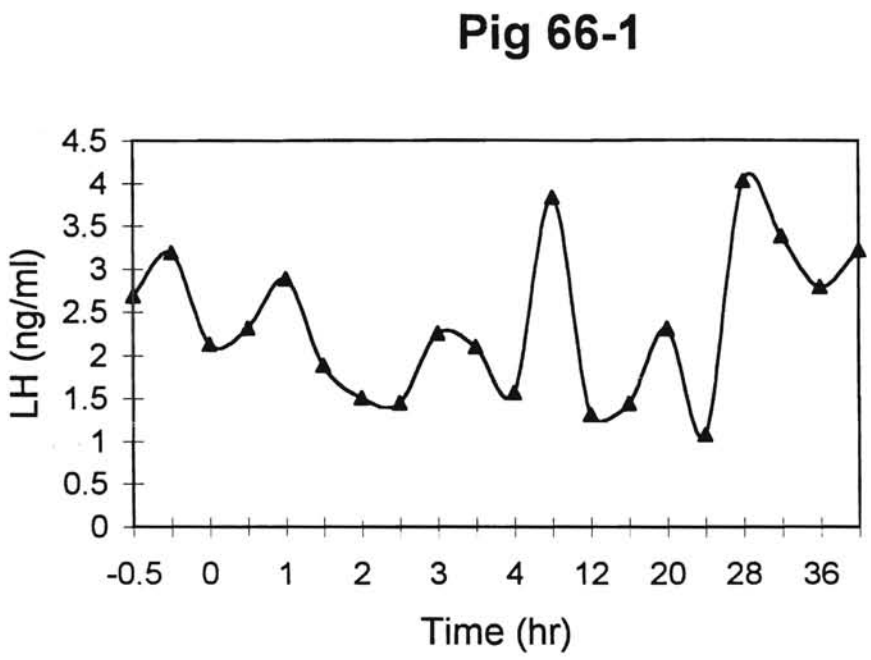
[3]Mid-cycle serum progesterone concentrations sampled on days 9 - 13 post-treatment.

[4]Numer of days from treatment until the next observed period of behavioral estrus.

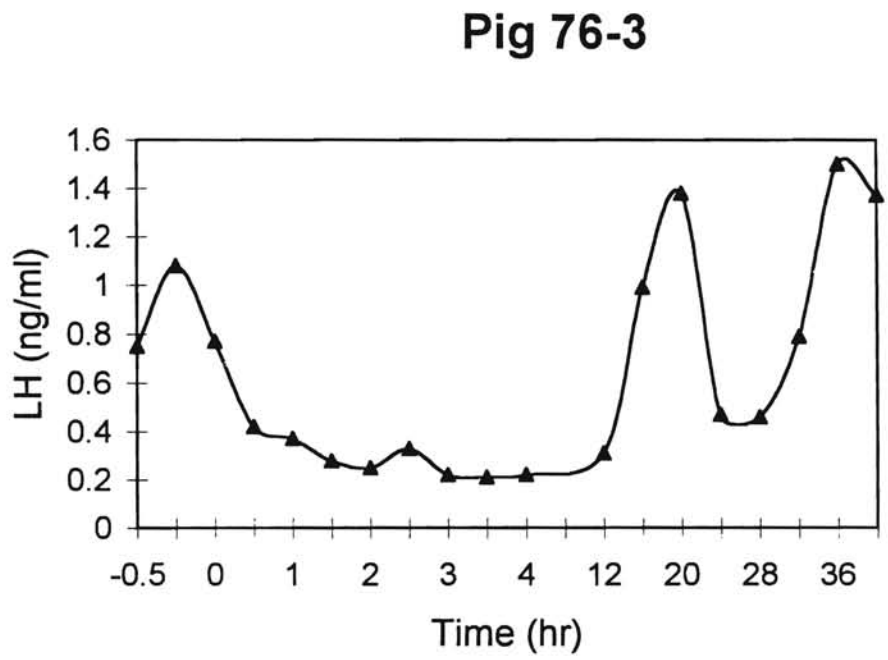
[5]Number of unovulated follicles at the time of second surgical evaluation.

Figure 2. Serum concentrations of LH in barrows treated with 10 μg/kg BW (A), 50 μg/kg BW (B) or 250 μg/kg BW (C) of GnRH antagonist. The antagonist was injected i.m. at time 0.

A


Pig 66-1
LH (ng/ml)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
0
1
2
3
4
12
20
28
36
Time (hr)

B


Pig 76-3
LH (ng/ml)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.5
0
1
2
3
4
12
20
28
36
Time (hr)

C

Pig 46-4

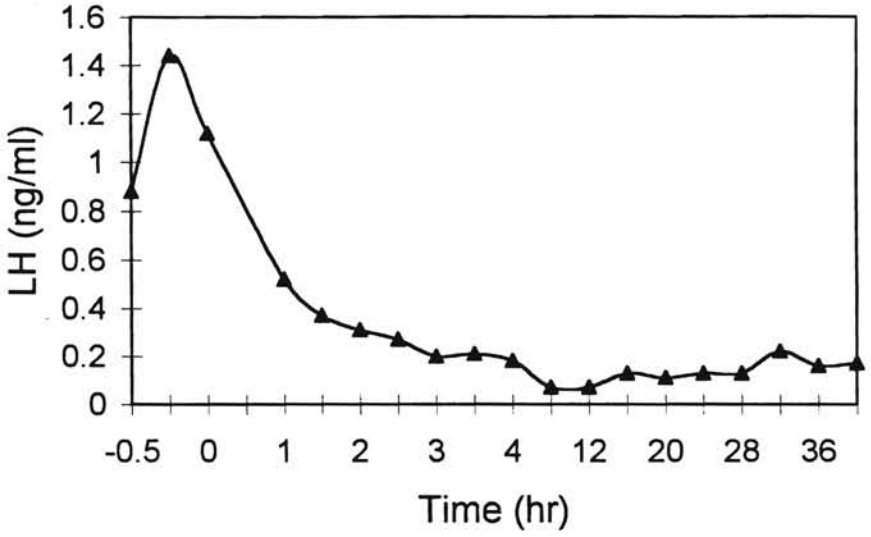
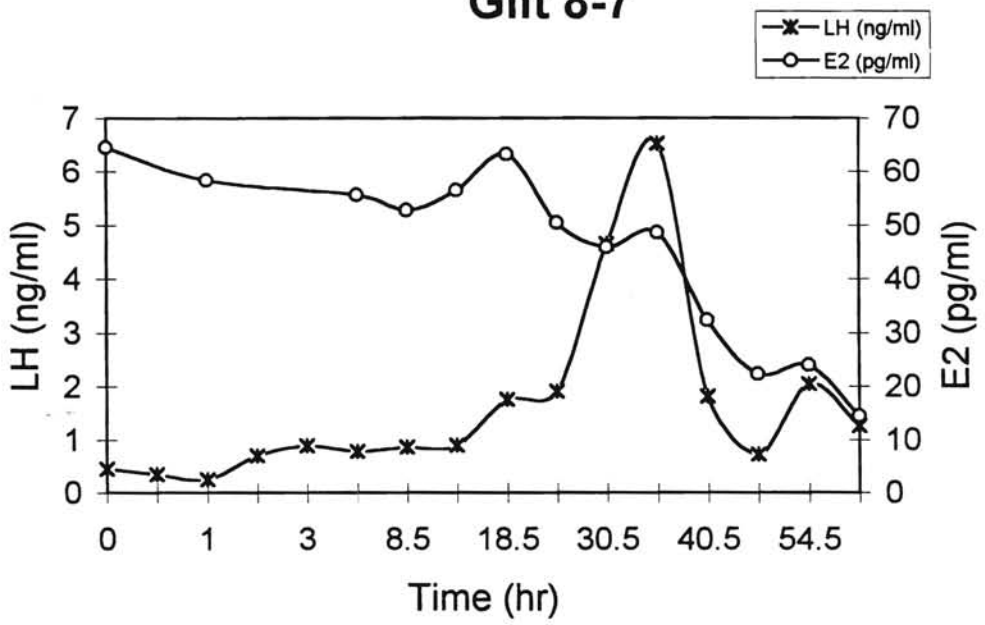
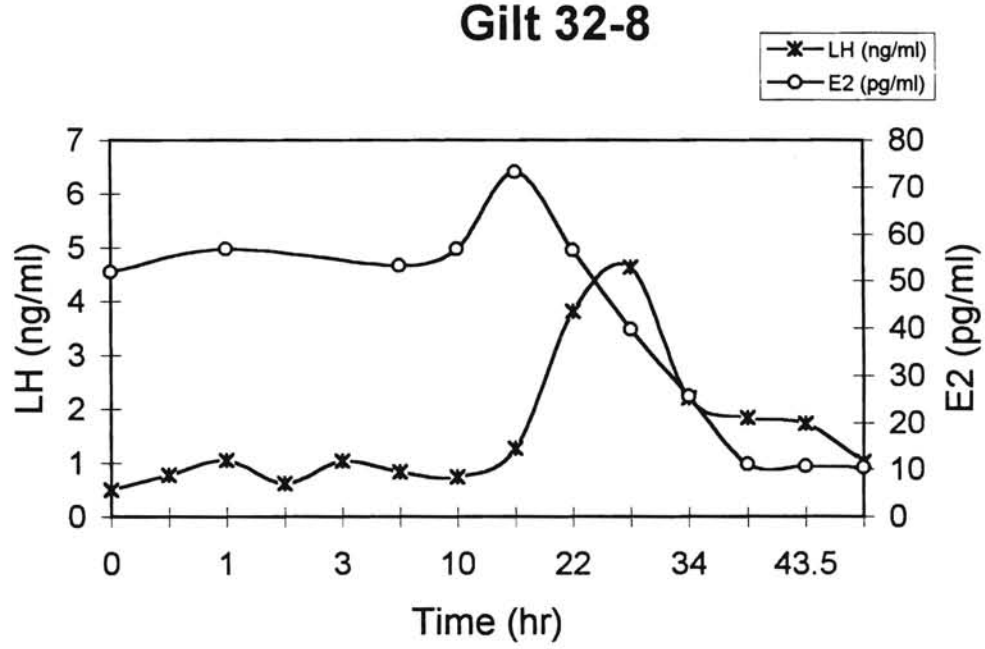


Figure 3. Serum concentrations of LH and E2 in control gilts (A, B, C & D) injected i.m. with saline (10 ml). Time 0 is onset of standing estrus.

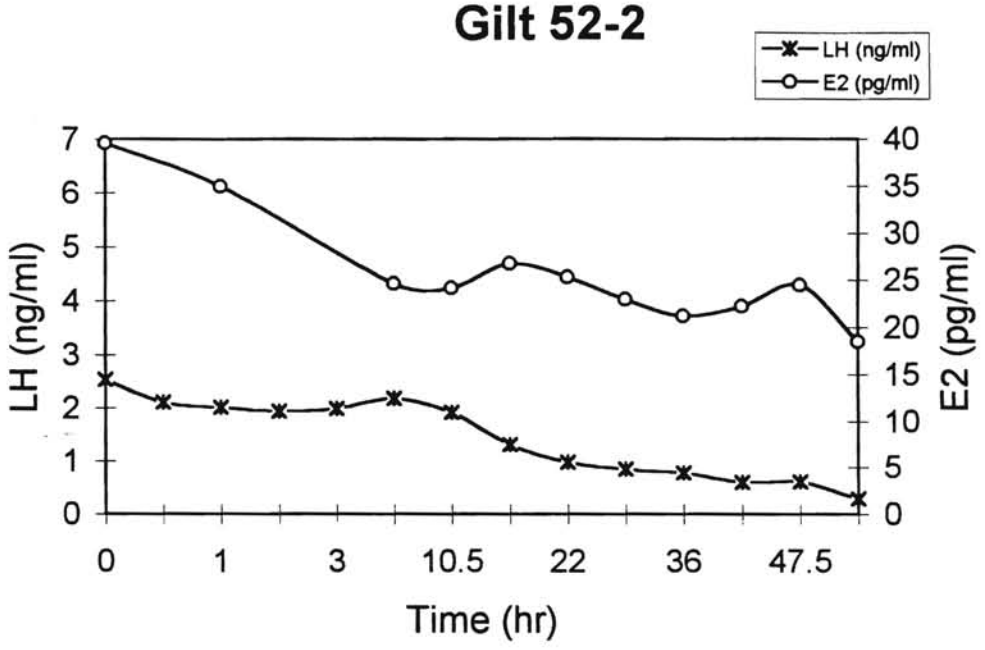
A


Gilt 8-7
LH (ng/ml)
E2 (pg/ml)
LH (ng/ml)
E2 (pg/ml)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
0
1
3
8.5
18.5
30.5
40.5
54.5
Time (hr)

B


Gilt 32-8
LH (ng/ml)
E2 (pg/ml)
LH (ng/ml)
E2 (pg/ml)
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
70
80
0
1
3
10
22
34
43.5
Time (hr)

C


Gilt 52-2
LH (ng/ml)
E2 (pg/ml)
LH (ng/ml)
E2 (pg/ml)
0
1
2
3
4
5
6
7
0
5
10
15
20
25
30
35
40
0
1
3
10.5
22
36
47.5
Time (hr)

D

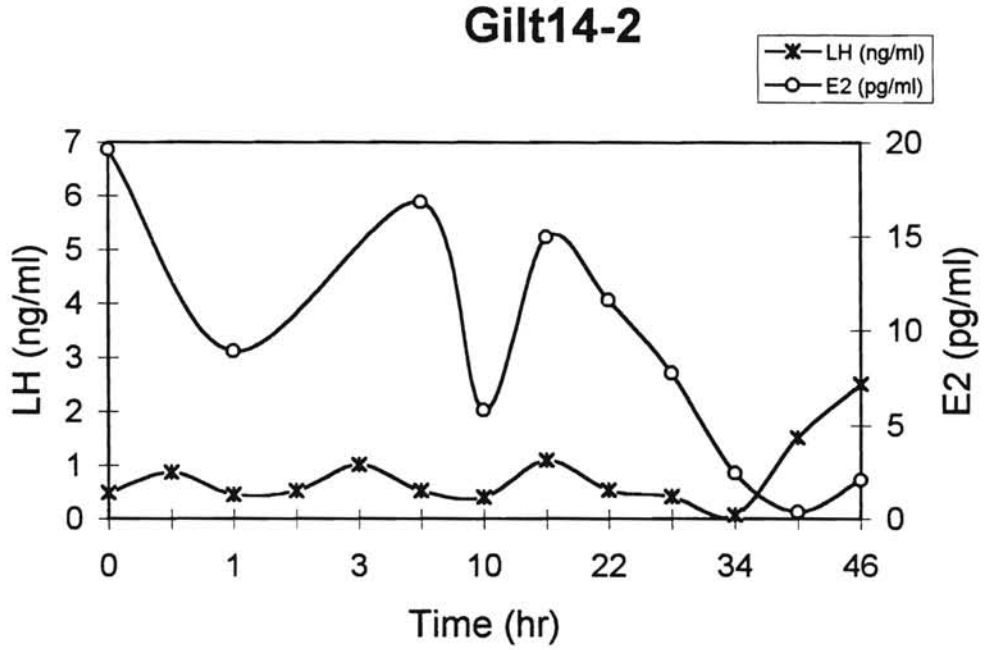
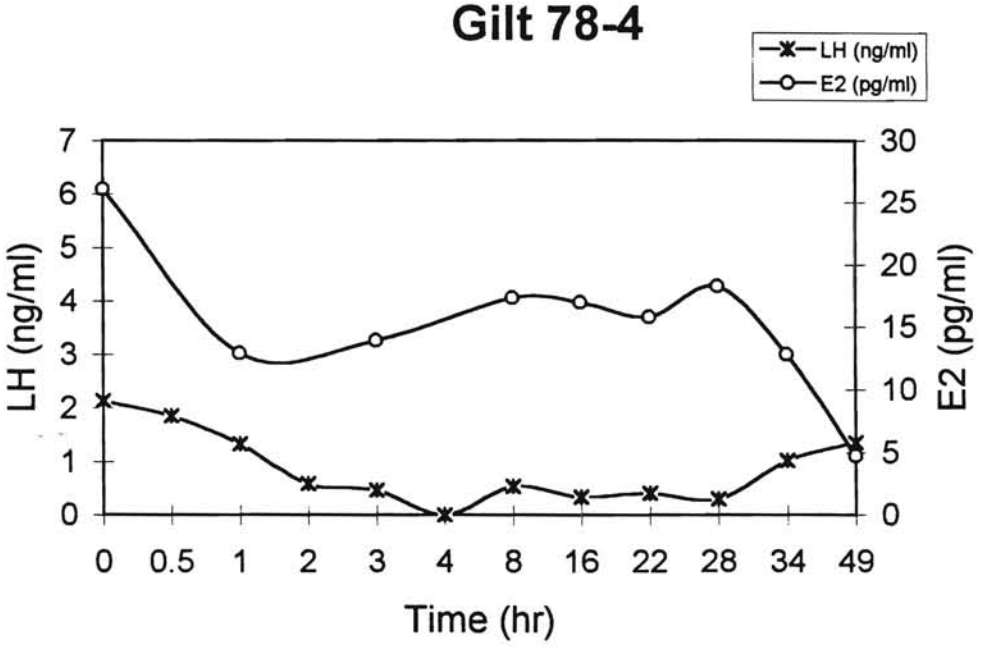
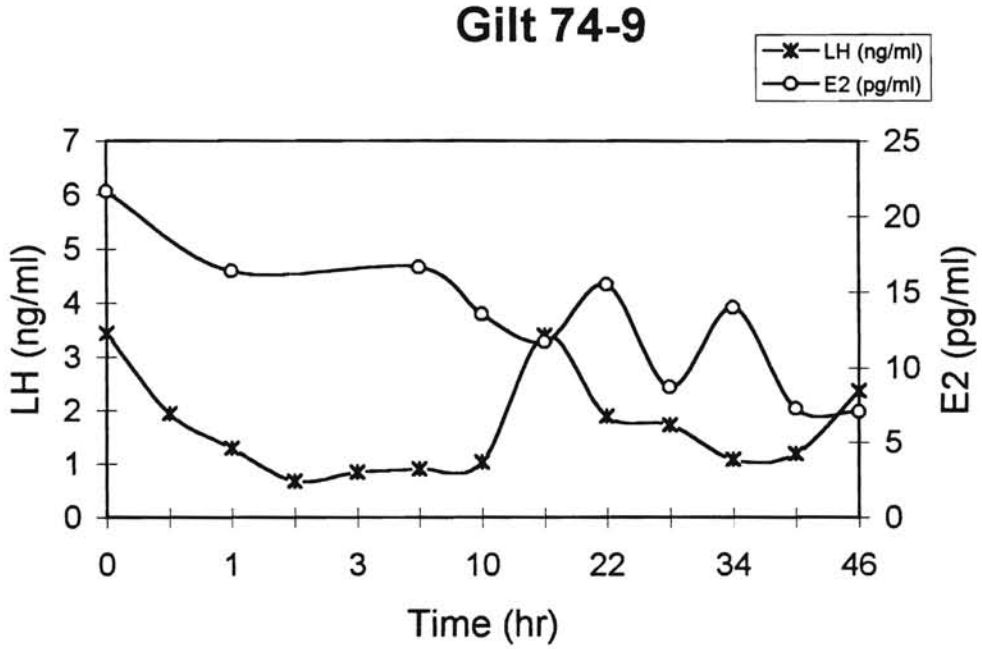

Gilt14-2
LH (ng/ml)
E2 (pg/ml)
LH (ng/ml)
E2 (pg/ml)
0
1
2
3
4
5
6
7
0
5
10
15
20
0
1
3
10
22
34
46
Time (hr)

Figure 4. Serum concentrations of LH and E2 in gilts treated with 100 μg/kg BW (A & B) or 200 μg/kg BW (C) of the GnRH antagonist. Time 0 is the onset of standing estrus and the time of i.m. injection of antagonist.

A


Gilt 78-4
LH (ng/ml)
E2 (pg/ml)
LH (ng/ml)
E2 (pg/ml)
0 1 2 3 4 5 6 7
0 5 10 15 20 25 30
0 0.5 1 2 3 4 8 16 22 28 34 49
Time (hr)

B


Gilt 74-9
LH (ng/ml)
E2 (pg/ml)
LH (ng/ml)
E2 (pg/ml)
0 1 2 3 4 5 6 7
0 5 10 15 20 25
0 1 3 10 22 34 46
Time (hr)

C

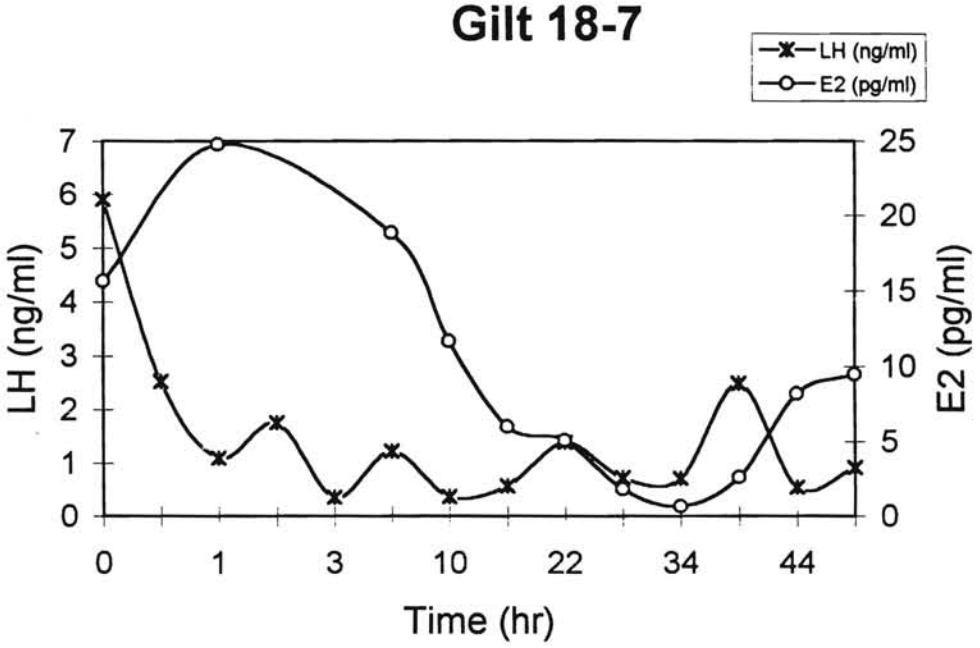
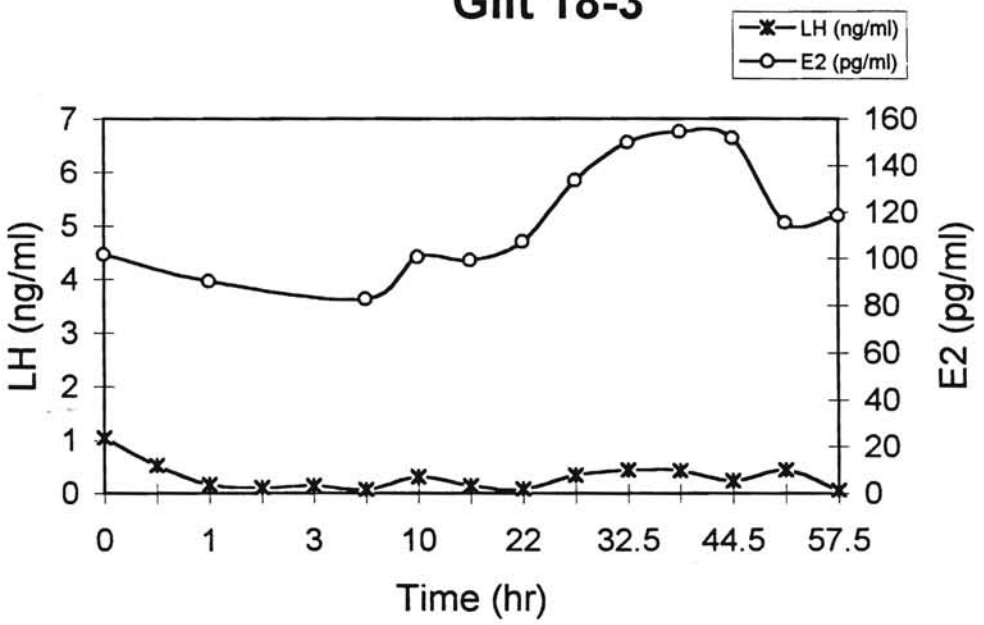
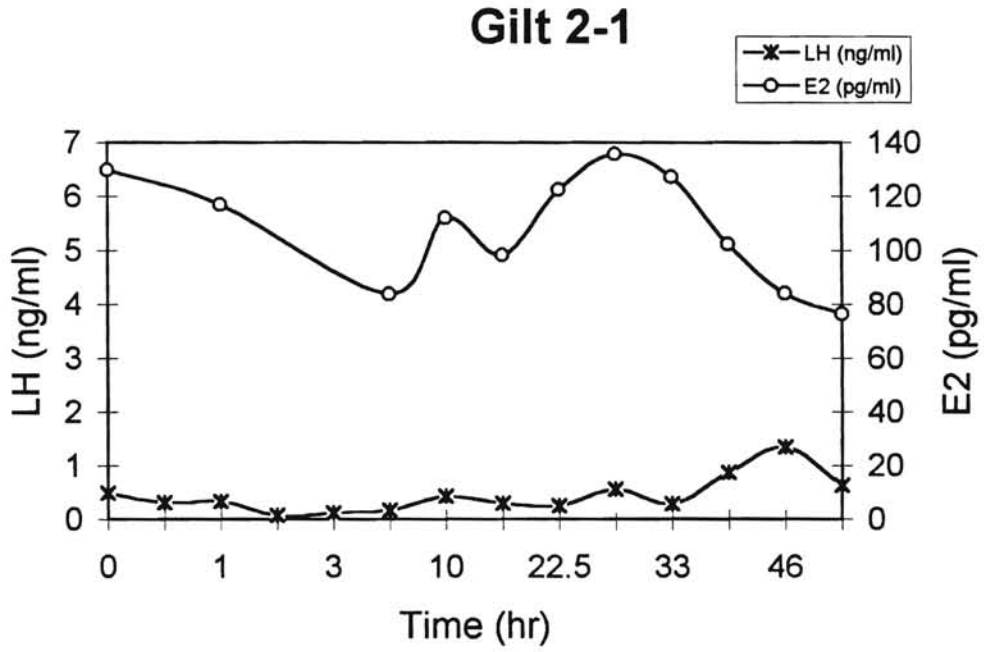

Gilt 18-7
LH (ng/ml)
E2 (pg/ml)
LH (ng/ml)
E2 (pg/ml)
0
1
2
3
4
5
6
7
0
5
10
15
20
25
0
1
3
10
22
34
44
Time (hr)

Figure 5. Serum concentrations of LH and E2 in GnRH2 gilts treated with 200 μg/kg BW of the GnRH antagonist 8 hours prior to standing estrus. Time 0 is the time of i.m. antagonist injection.

A


Gilt 18-3
LH (ng/ml)
E2 (pg/ml)
LH (ng/ml)
E2 (pg/ml)
0 1 2 3 4 5 6 7
0 20 40 60 80 100 120 140 160
0 1 3 10 22 32.5 44.5 57.5
Time (hr)

B


Gilt 2-1
LH (ng/ml)
E2 (pg/ml)
LH (ng/ml)
E2 (pg/ml)
0 1 2 3 4 5 6 7
0 20 40 60 80 100 120 140
0 1 3 10 22.5 33 46
Time (hr)

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