# Abstract

Roski, Kara Howes.  Ovulatory and reproductive characteristics of sows treated with an intravaginal GnRH agonist gel.  (Under the direction of William L. Flowers.)

This study was designed to test the reproductive and ovulatory characteristics of sows treated with Ovugel® (EIEICO, Radnor, PA), a gel containing a GnRH agonist (Triptorelin) administered intravaginally.  The treatment groups received 100μg of GnRH agonist intravaginally in varying viscosities of the gel, 0.6%(n=12), 0.9%(n=12), 1.2%(n=12), and 1.5%(n=12) respectively.  A positive control group (n=11) received saline containing 100μg of GnRH agonist while the control sows (n=12) received a vehicle of the 1.2% gel and at 96h post weaning.  Jugular cannulas were placed 48 hours before administration of the treatment.  Blood samples were taken every 6 hours before treatment, then every 2 hours for the first 12 hours after treatment, then every 6 hours for the next 18 hours.  Estrus detection occurred every 6 hours through the entire study.  Real time ultrasonography was conducted every 4 hours to determine the time of ovulation.  Control sows were bred based on the onset of estrus and treatment sows were bred at +8 and +32 h after the gel was administered.  All data was analyzed using SAS and the proc GLM procedure.

| Treatment | Saline + GnRH | Control | 0.6% + GnRH | 0.9% + GnRh | 1.2% + GnRH | 1.5% + GnRH |
|---|---|---|---|---|---|---|
| Ovulation time (h) | 46.5±1.2 | 43.8±5.4 | 43.8±2.0 | 41.2±3.3 | 43.5±1.8 | 44.5±1.5 |
| Estrus length (h) | 45.8±3.4 | 45.6±3.8 | 49.0±3.8 | 47±3.2 | 46.9±3.9 | 55.1±3.9 |
| LH surge (0-30h post treatment) | 7/10 | 5/8 | 7/10 | 9/11 | 8/10 | 8/9 |
| Farrowing Rate | 8/8 | 9/9 | 11/12 | 10/12 | 11/12 | 10/11 |
| Litter Size | 12.9±0.7 | 9±3.1.2 | 11±1.3 | 11.6±1.0 | 11±0.5 | 10.6±1.4 |

There were no differences in treatments in ovulation time from the onset of estrus or administration of Ovugel® (p=0.28), estrus length (p=.60), farrowing rate (p=0.80), litter size (p=0.35), or LH surge (p=0.49). However, variation associated with ovulation time was significantly reduced (p=0.01) for saline, 1.2%, and 1.5% treatments compared with controls. The duration of estrus was greater for the 1.5% gel with the triptorelin than the sows receiving only the 1.2% gel (p=0.04). The intravaginal absorption of GnRH in Ovugel® or saline along with a timed insemination program is an effective method to reduce the interval over which ovulation occurs without overtly decreasing fertility.

KEY WORDS: ovulation, GnRH agonist, swine

Ovulatory and reproductive characteristics of sows treated with an intravaginal GnRH agonist gel


By



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Chair of Advisory Commitee



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**<u>Biography</u>**


      Kara Roski was born on July 28, 1979 to Kim Kercheval and Richard Roski in Cleveland, Ohio.  Her parents already had one daughter, Katie.  When she was two, her mother and sister moved to Carmel, Indiana.  Her father re-married Debbie Roski and blessed Kara with another sister, Courtney, and brother, Justin.  The author attended grade school and high school in Carmel.

      In August of 1997, Kara followed in many of her family's footsteps and began working on her bachelor's degree in Animal Science at Purdue University in West Lafayette, Indiana.  After completing her bachelor's, Kara moved to Raleigh, North Carolina in August of 2002.  Here she entered a graduate program at North Carolina State University and is pursuing her Master of Science degree in Animal Science.

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# Introduction

Artificial insemination is used in over 90% of the swine industry. Most farms check their sows for estrus one or two times per day. If the sow shows the standing estrus reflex, then they are bred by artificial insemination for as many days as they stand. Therefore each sow is bred two or three times. Most of the problems associated with breeding are inaccurate detection of estrus and poor insemination techniques and timing. In order to reduce these errors, timed insemination protocols have been suggested. Moreover each estrus check and insemination requires labor. As a result, breeding programs in the swine industry are one of the greatest expenses on farms. It is important to understand the reproductive cycle in swine in order to properly time inseminations and develop ways to reduce labor costs.

## Literature Review


## Estrous Cycle


Gilts reach puberty and are capable of sexual reproduction between 200 and 210 days of age, but it can occur as early as 150 days with exposure to a boar (Hughes, 1982). Attainment of puberty depends on numerous characteristics including weight, season and sex. Once a gilt reaches puberty she begins exhibiting normal estrous cycles. The estrous cycle of the sow can be divided into three phases: the follicular phase (also called proestrus), the luteal phase (also called diestrus), and estrus (the period of sexual receptivity). The entire estrous cycle of the sow is between 17 and 25 days (Dial and Britt, 1986) with an average of 21 days (Dial and Britt, 1986, Van de Wiel et al., 1981).

If day 0 is the first day of standing estrus then the luteal phase is from day 4 to 15 of the cycle (Dial and Britt, 1986, Prunier et al., 1987). During this time, functional corpus lutea (CL) are present on the ovary. The corpus luteum develops after the follicle has ovulated and produces progesterone. Progesterone concentrations begin to increase two days after the end of estrus and reach maximum concentrations of 28 to 48 ng/ml by day 7 to 13 of the estrous cycle (Henricks et al., 1972). Similarly, Van de Wiel et al. (1981) reported that progesterone concentrations began to increase 27 to 30 hours after estrus and peaked on day 5 to 7 with concentrations ranging from 28.3 to 30 ng/mL. Peak concentrations persist for 2 to 3 days and then begin to decrease around day 15 to 16 of the estrous cycle (Henricks et al., 1972). Also during the luteal phase, LH secretion is characterized by a pattern of low frequency and high amplitude pulses (Foxcroft and Van de Wiel, 1982) with peak concentrations of 3.5 ng/mL (Van de Wiel et al., 1981).

Follicles are less than 4 mm in size at this time until about day 9 of the cycle. By day 12 of the estrous cycle, the size and number of follicles has increased (Erb, 1971). If pregnancy is not established, progesterone concentrations will decrease due to luteolysis of the CL (Dial and Britt, 1986). Prostaglandin F2$\alpha$ (PGF2$\alpha$) is secreted from the uterine endometrium in an endocrine fashion traveling through the bloodstream to the ovary to cause luteolysis (Bazer et al., 1982). Kotwica et al. (1990) reported that the rise in PGF2$\alpha$ was accompanied by an increase in oxytocin concentrations suggesting that oxytocin may also play a role in luteolysis.

Progesterone acts in a negative feedback loop blocking the estrogen-induced pituitary secretion of LH (Dial and Britt, 1986). Progesterone concentrations are below 1 ng/mL in gilts until 27-30 hours after the maximum LH concentration (Van de Wiel et al, 1981) thereby releasing the negative feedback on LH secretion. The frequency of LH pulses increases and stimulates follicular growth. The follicular phase of the cycle is classified by the growth and development of follicles. Prior to the beginning of the follicular phase, the follicles grow from primordial follicles which contain an oocyte that is arrested in prophase I of meiosis surrounded by a single layer of undifferentiated granulosa cells to a primary follicle classified by the differentiation of granulosa cells. Multiple layers of granulosa cells form as well as the formation of theca cells in the secondary follicle. Follicle stimulating hormone (FSH) increases causing an antrum to form and the maturation of the Graafian or preovulatory follicle (Reeves, 1987). FSH also acts in conjunction with LH to cause the production of estrogen from the follicle (Reeves, 1987) through the two-cell theory of follicular steroidogensis (Foxcroft and Hunter, 1985). Hafez (1987) describes the process of steroidogenesis as beginning with

LH binding the theca cells' membrane and stimulating the conversion of cholesterol into androgens through the delta 4 or delta 5 pathway. The theca cells lack the enzyme aromatase that is responsible for converting androgens to estrogens. Therefore the androgens produced by the theca cells act as a precursor in the granulosa cells, which produce aromatase, for the production of estrogen. Granulosa cells can convert cholesterol into progestins in response to FSH, but lack the enzyme 17α-hydroxylase so are therefore unable to produce estrogen alone. In the presence of FSH and estrogen, LH receptors are recruited on the surface membrane of the granulosa cell causing it to become LH dependant, and reach a maximum number at day 20 of the estrus cycle (Foxcroft and Hunter, 1985).

During the mid to late follicular phase, follicles on the ovary are greater than 6 mm in size (Babalola and Shapiro, 1988). Estrogen produced by the follicles acts on the hypothalamus to cause the release of GnRH and subsequently LH and FSH. Estrogen concentrations begin increasing significantly 2 days prior to estrus (Henricks et al., 1972). Estrogen concentrations continue to increase until they hit a maximum concentration of 32.1 to 56.4 ng/mL at 8 to 15 hours before the pre-ovulatory surge of LH (Van de Wiel et al., 1981). Peak estrogen concentrations are responsible for estrus behavior (Dial and Britt, 1986). Estrogen concentrations actually decrease on the day of estrus (Henricks et al., 1972, Van de Wiel et al., 1981). Prior to estrus, prolactin concentrations are between 5 and 14 ng/mL until two hours after the maximum estrogen concentration. After the peak estrogen secretion, prolactin concentrations increase to a maximum of 28.3 to 32.0 ng/mL and remain high for about 14 days (Van de Wiel et al.,

1981).    Van de Wiel et al. (1981) suggest that prolactin may also play a role in estrous behavior.

Day 0 of the estrous cycle is classified as the first day females exhibit the standing reflex.  Estrus is the phase of the cycle where the sow is sexually receptive.  Estrus lasts $60 \pm 11$ hours (Soede et al., 1995) or between 24 and 96 hours (Bazer et al., 1982, Soede and Kemp, 1997).  The rise of estrogen between days 18 and 20 of the estrous cycle triggers the release of GnRH from the hypothalamus that in turn causes a pre-ovulatory release of LH from the pituitary (Foxcroft and Van de Wiel, 1982, Dial and Britt, 1986).  Hansel and Convey (1983) suggested that this elevated estrogen concentration could increase the ability of the hypothalamus to prime the pituitary gland for gonadotropin secretion causing a greater amount of LH and FSH to be released.  This preovulatory LH surge takes place on the day of estrus (Henricks et al., 1972, Van de Wiel et al., 1981, Kemp and Soede, 1997).  The initial rise in LH occurs 1-3 hours prior to the estrogen maximum concentration (Van de Wiel et al., 1981).  Van de Wiel et al. (1981) reported that the maximum LH concentrations were between 4.2 and 5.9 ng/mL and were seen after 12 hours from the start of the surge.  Prunier et al. (1987) reported that the surge lasted 13 to 20 hours with maximum LH concentrations were 10.3 to 23.1 ng/mL.  Hansel and Convey (1983) as well as Dial and Britt (1986) reported that there are inconsistencies in the data about when the LH surge takes place relative to the onset of estrus behavior with a range of 12 hours before to 12 hours after.

However there is a fairly consistent time interval between the peak of the LH surge and the time at which ovulation takes place.  Estimates of 30 to 48 hours have been reported (Foxcroft and Van de Wiel, 1982, Soede et al., 1994, Liptrap and Raeside,

1966).  Although there is variation between sows in the duration of estrus, on average, ovulation occurs at 70% of the way through estrus (Soede and Kemp, 1997).  Follicles capable of ovulation are between 5 to 12 mm in size (Dial and Britt, 1986).  Immediately following ovulation, estrogen concentrations decrease and there is a surge in FSH that lasts 2 to 3 days (Dial and Britt, 1986).  Once ovulation occurs the remaining follicular cells become a functional CL and begin to produce progesterone.

## **Pregnancy**

If the sow becomes pregnant, the blastocyst produces estrogen, which maintains the functioning CL (Bazer et al., 1982).  This estrogen production around day 10 to 12 post coitum is a signal for maternal recognition of pregnancy in the sow (Flint et al., 1978, Gadsby et al., 1980).  Estrogen may interact with prolactin to cause the PGF2$\alpha$ to switch to an exocrine fashion and be secreted into the uterine lumen where it is then broken down (Bazer et al., 1982, Bazer et al., 1989, Dial and Britt, 1986, Geisert et al., 1990).  The porcine species is litter bearing and, therefore, maintenance of the CL depends on the number of embryos present in the uterus.  The sow requires at least 4 live embryos to be present to maintain pregnancy (Erb et al., 1971, First et al., 1982, Dzuik, 1985, Dial and Britt, 1986).  In addition to a specific number of embryos, the number of CLs is also important.  At least 4-6 CLs must be present to produce adequate amounts of progesterone to maintain pregnancy (First et al., 1982).

The gestation length of the sow is between 112 to 116 days (First et al., 1982). Uterine contractions remain quiet due to the high levels of progesterone (Dial and Britt, 1986). During late pregnancy, a few key hormones interact to maintain pregnancy and

prepare the mammary gland for lactation.  Progesterone concentrations are between 6 and

12 ng/mL and then decrease just before parturition (Ash et al., 1975).  This decline in

progesterone 1 to 2 days before parturition causes estrogen levels to increase (Ash et al.,

1975, First et al., 1982). Estrogens have direct effects on the growth of the uterine

endometrium as well as priming effects on the mammary gland to increase its sensitivity

to prolactin and on the cervix to increase its sensitivity to relaxin (Reeves, 1987).  This

decrease in progesterone also triggers the production of PGF2$\alpha$ that in turn causes the

release of relaxin, prolactin and oxytocin (First et al., 1982). .  Prolactin acts as a

luteotropic substance as well as inducing maternal behavior in the sow (Reeves, 1987).

Relaxin is a hormone responsible for relaxing the cervix to allow for the fetuses to pass

the cervix, while oxytocin is responsible for the milk ejection and uterine contractions

(First et al., 1982).


## Lactation


Once parturition is complete, lactation begins.  Lactation can be classified as a

period of anestrous behavior lasting 2 to 6 weeks as long as suckling patterns are normal

(Britt, 1986).  Okrasa et al. (1989) found that each suckling event triggered hormonal

changes in the sow.  In early lactation, the levels of gonadotropins are low (Edwards,

1982, Dial and Britt, 1986, Rojkittikhun et al., 1993) probably due to the suckling

stimulus inhibiting GnRH secretion (Varley, 1986).  Therefore, the ovary contains mainly

small follicles (< 4 mm) and a few medium follicles (Britt et al., 1985, Killen et al.,

1992).  The suckling induced inhibition of follicular development is controlled, in part,

by opiods (Quesnel and Prunter, 1995).  An initial and immediate increase in oxytocin

occurred after each suckling event during early lactation.  They found that in 90% of all suckling events prolactin increased and in 64% of the suckling events corticosteroids increased.  The average concentration of LH and the number of pulses of LH increases beginning on day 10 – 20 of lactation (Rojkittikhun et al., 1993) to stimulate follicular growth.

## Weaning

Weaning causes many changes in the hormonal profiles of the sow.  Most sows will exhibit an increase in follicular growth within the first 6 hours following weaning (Edwards, 1982, Britt et al., 1985, Dial and Britt, 1986, Killen et al., 1992).  This culminates in estrus and ovulation within 3 to 8 days after weaning (Edwards, 1982, Dial and Britt, 1986, Varley, 1986).   Immediately following the removal of the litter, the sow exhibits an increase in GnRH within 60 hours (Cox and Britt, 1982, Quesnel and Prunier, 1995, Edwards, 1982), most likely associated with the removal of the inhibitory suckling stimulus (Britt et al., 1985, Varley, 1986).  This increase in GnRH causes a subsequent increase in basal LH concentration (Cox and Britt, 1982, Varley, 1986, Edwards and Foxcroft, 1983a) that lasts 1 to 2 days (Edwards, 1982).  Dial and Britt (1986) also observed an increase in LH pulse frequency.  Foxcroft et al. (1987) reported that the frequency rose from 4.6 episodes prior to weaning to 9.2 to 9.4 episodes during 12-hour sampling periods following weaning.  In contrast, FSH exhibited a much smaller increase after weaning (Varley, 1986), or none at all (Edwards, 1982, Edwards and Foxcroft, 1983).   If one is observed, follicle size increases to greater than 5 mm in response to these hormonal changes (Cox and Britt, 1982).

Due to the follicular growth and increased LH pulse frequency, estrogen levels begin to rise in the sow (Dial and Britt, 1986, Varley, 1986). Edwards (1982) reported that this increase in estrogen concentrations might be lower in the weaned sow than in cyclic sows. Prolactin concentrations decrease after weaning for about 12 hours (Edwards, 1982, Foxcroft et al., 1987). Prolactin concentrations increase at the time of estrus in the lactating sow, but the weaned sow does not show the same increase (Edwards, 1982).

Some research has been done to examine the effects of a shortened lactation length on post-weaning estrus characteristics. The swine industry is interested in this because a shorter lactation length would allow for a shorter wean-to-first service interval for the sow and subsequently more litters per year. This would decrease the number of the sow's nonproductive days. Edwards (1982) compared 3-week lactation lengths to 5-week lactation lengths and found a decrease in gonadotropin secretion. LH surge peak and total concentration was lower with the 3-week lactations compared to the 5-week lactations. Sows weaned after a 5-week lactation exhibited LH and FSH surges, as where, the sows that were weaned after a 3-week lactation did not. Edwards and Foxcroft (1983a) observed that a decreased lactation length decreased LH surge concentrations without altering ovulation or wean-to-estrus interval. Koketsu and Dial (1997) reported that lactation lengths of 1 to 7 days coincided with extended wean-to-conception intervals, but lactation lengths greater than 19 days coincided with shorter wean-to-conception intervals. This was similar to earlier studies where a reduced lactation length from 42 days to 7 days increased the wean-to-estrus interval from 6.1 days to 8.2 days without altering ovulation rates (Varley and Cole, 1976a). Weitz et al.

(1994) studied the onset of heat after weaning after a 20 day lactation length and found that wean to estrus interval influenced the length of estrus. Sows that came into estrus early (about 3 days after weaning) were in estrus for 70 hours or greater compared with the sows that came into estrus later (about 5 days after weaning) which were in estrus for less than 60 hours.

Varley and Cole (1976a) also reported a reduction in the number of viable embryos present and an increase in embryo mortality from 17.3% to 40.4% with a decrease in lactation length from 42 days to 7 days. A shortened lactation length can also decrease litter size at the next parity (Varley and Cole, 1976b, Koketsu and Dial, 1997). It might also be important to note that sows in their first parity exhibited longer wean to first service and wean to conception intervals (Koketsu and Dial, 1997).

Administration of exogenous hormones around or at the time of weaning has also been studied as a management practice to decrease the time from weaning-to-conception. Estienne and Hartsock (1998) studied the effects of administering PG 600 (a substance containing PMSG and hCG that mimic the effects of LH and FSH in the sow) to sows at weaning. They found that the number of sows that returned to estrus within 7 days increased from 82.9% to 97.1%. The sows treated with PG 600 also had larger litter sizes but a decreased farrowing rate.

This interval from weaning to ovulation can also depend on the parity and body condition of the sow. Bracken et al. (2003) reported that sows with a low body condition score had smaller diameter follicles present on the ovary at 3 days post weaning compared to sows of a higher body condition score. They also reported that 1st parity sows showed a longer interval from weaning to ovulation than sows of higher parities.

**Response to Mating**


Sows exhibit an endocrine response to mating. Exposure to a boar will elicit an increase in cortisol concentrations from $16.1 \pm 11.6$ ng/ml to $46.6 \pm 9.6$ ng/ml (Kotwica et al., 2002). This increase in cortisol was maintained at $48.8\pm9.8$ ng/ml until 30 minutes after mating. Oxytocin concentrations also are affected by mating stimuli. Claus and Schams (1990) measured oxytocin concentrations from 1 hour before to 5 hours after mating. They found that mating stimulated the release of oxytocin concentrations from 1 pmol/l to a maximum of $42.0\pm5.1$ pmol/l. The maximum concentration occurred 2 minutes after ejaculation and the rise in oxytocin lasted for 40 minutes. Kotwica et al. (2002) determined that an increase in oxytocin was followed by an increase in cortisol concentrations that could be enhanced with the administration of estradiol and progesterone.

Claus (1990) studied seminal components of boars and their subsequent effects in the reproductive tract of the sow. The ejaculate of the boar is rich in estrogens. Therefore, Claus used saline containing physiological amounts of estrogens to inseminate the sow and found a 2.5 fold increase in myometrial contractions. In addition, immediately after these estrogens were administered, an increase in PGF2$\alpha$ was observed. The infusion of estrogens increased peripheral estrogen concentrations. Ziecik et al. (1981) studied the effects of natural versus artificial matings on LH release. These researchers found an increase in plasma LH concentrations in naturally mated sows on

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the morning after the first day of estrus (2.75±.54 ng/ml) compared to sows mated with whole semen (0.89±0.1 ng/ml), sows mated with seminal plasma (1.02±0.13 ng/ml) or non mated sows (0.92±0.11 ng/ml). Kirsch et al. (1985) also studied the effects of natural mating versus artificial insemination on LH concentrations and LH surge characteristics. The mean plasma LH concentration did not differ between the naturally mated and inseminated sows. The LH surge characteristics of peak and maximum concentration also did not differ between the naturally and artificially mated sows. The length of the LH surge was longer for naturally mated (67 hours) and non-mated control sows (87 hours) than for the artificially inseminated sows (45 hours). The total concentration of LH was decreased in the artificially inseminated sows due to smaller area under the curve.

Mating also plays a role in a number of species to induce ovulation. These induced ovulators include cats, ferrets, camels and llamas to name a few. Mating can also have effects on the time at which ovulation takes place and the duration of ovulation in sows. Signoret et al. (1972) studied the effects of mating on ovulation in 59 gilts that had been receiving methallibure orally for 21 days to synchronize estrus. The gilts were mated at the first standing estrus and then again 6 hours later. The median time of ovulation occurred 3.9 hours earlier in animals that were bred twice than the animals that were not mated. The duration of ovulation was also reduced in sows that were mated by 2.8 hours. This suggests that ovulation is advanced in response to mating stimuli when bred in a 6-hour time interval. This contradicts a study by Zimmerman and Naber (1971) in which ovulation was not advanced in sows bred at 12 hours after the first standing estrus was observed.

## Displacement of Homeostasis

There are many situations in which the hormones of the hypothalamic-pituitary-adrenal axis are released in the sow including exposure to a boar, mating, housing conditions, social hierarchy, heat or cold stress, and handling by people. In these situations, the hypothalamus releases opiods and the hormone corticotropin-releasing hormone which acts on the pituitary gland causing a release of adrenocorticotropic hormone (ACTH). ACTH travels to the adrenal cortex and causes the release of cortisol. Baldi et al. (1989) found that the release of ACTH from the pituitary caused an increase in the plasma cortisol concentration. Becker et al. (1985) used heat, electric shock and confinement as stressors on pigs and found that cortisol was a good indicator of stress. ACTH concentrations have also been used as an indicator of stress or to induce a stress response in pigs. Corticotropin releasing hormone (CRH) can also be used to induce a stress response. Johnson et al. (1994) injected porcine and murine CRH intracerebroventricularly and found an increase in ACTH along with hyperactivity of the sow and an increase in vocalizations of the sow. Lang et al. (2004) found an increase in plasma cortisol concentrations in two out of four sows following injection of CRH.

Stimulation of the hypothalamic-pituitary-adrenal axis also has effects on other organs and systems of the body. Increases in ACTH and the subsequent rise in cortisol are accompanied by an increase in prostaglandin $F2\alpha$ metabolite and progesterone (Mwanza et al., 2000a, Razdan et al., 2002). A similar rise in cortisol, prostaglandin $F2\alpha$ metabolite and progesterone was seen by Mwanza et al. (2000b) after administration of ACTH. They also noted that estrogen concentrations did not increase when the

concentrations of corticosteroids rose. This increase in corticosteroids is also known to decrease the immune system of animals.  Johnson et al. (1994) saw a suppressed production of lymphocytes when 50 or 150 µg of CRH was administered.

A rise in corticosteroid concentrations can also play a role in the reproductive processes of the pig.  Cortisol concentrations increased when sows were removed from their pens and came in olfactory contact with a boar (Kotwica et al., 2002).  Pearce and Hughes (1987) discovered that full exposure to a boar would induce a significant rise in cortisol.  Injection of ACTH beginning on day 14 of the estrous cycle until the end of standing estrus causes a delay in the peak concentration of estrogen as well as a delay in estrous behavior (Liptrap, 1970).  Parturition is also associated with an increase in the concentrations of total and free cortisol (Whitley et al., 1984).   The free cortisol is not bound to a  carrier protein and therefore able to enter cells and have a biological effect.

In vitro studies have also shown that cortisol can play a role in the production of gonadotropins.  Li (1987) incubated pituitary cells with cortisol or ACTH then added GnRH to measure the LH response.  Cortisol was shown to inhibit the rise in LH induced by estrogen and subsequently the GnRH-induced LH response.  Barb et al. (1982) found that treating gilts with ACTH blocked the LH surge and therefore blocked ovulation. Lang et al. (2004) found that treating sows with ACTH before the LH surge had begun appeared to decrease the peak LH concentration, but administration of ACTH after the surge had begun had no effect.  Therefore, the effects of ACTH may depend on the stage of the estrous cycle.

The stage of follicular development also can impact the effects of cortisol.  ACTH given in the luteal phase alters IGF-1 as well as steroid hormone production in the ovary

(Viveiros and Liptrap, 2000). Cortisol does not seem to have a negative effect on oviductal transport rate (Razdan et al., 2002), oviductal pressure or number of ovum in the oviduct (Mwanza et al., 2000a). ACTH given in the follicular phase of the estrous cycle caused cystic follicles to occur in ovarectomized sows (Scholten, et al., 1978).

## Timing of Artificial Insemination

Artificial insemination catheters in the swine industry place the semen in the cervix of the sow. The sperm must then be transported from the cervix to the site of fertilization at the ampullary-isthmic junction of the oviduct. Success of insemination can be dependent on the success of sperm transport to the oviduct. Sperm first travel to the uterotubular junction, which is the area that connects the uterus to the isthmus of the oviduct. Here, some sperm come in contact with ciliated cells of the epithelium and form a sperm reservoir before ovulation takes place. Mburu et al. (1977) found that the sperm that bind to the epithelium were better equipped for fertilization than those that did not due to the maintenance of their plasma membranes. Sperm appear to remain quiescent at the uterotubular-isthmus junction for at least 36 hours until ovulation (Hunter, 1984). Ovulation appears to be a signal for sperm to be released from the cilia and travel to the ampullary-isthmic junction for fertilization (Mburu et al., 1977, Mburu et al., 1996, Rodrigues-Martinez et al., 2001). Rodrigeus-Martinez et al. (2001) suggested that the

sperm leave the reservoir after ovulation takes place as a result of capacitation and hyperactivation.  Hunter (1984) conducted an experiment in which sows were mated at the onset of estrus, and then the oviduct was isolated at 38h, 40h, and 42-44h after mating.  Fertilization rates were 5%, 40%, and 100%.  Artificial insemination protocols need to take into account the length of time that sperm need to mature and travel in the oviduct relative to ovulation.

When using artificial insemination, the success of the mating can depend on many factors concerning herd management.  Determination of the onset of heat, estrous length, wean-to-estrus intervals, and timing of ovulation are some of these factors.  It is important to coordinate these events properly in order to ensure a successful insemination.  Detection of estrus on the farm is a critical component of a fertile insemination.  Most farms use fence-line boar exposure along with the back-pressure test to determine the onset of heat.  It can be hard to determine the exact time of the beginning of estrus if the farm only heat checks the sows once a day.  The length of estrus can also play a role in the success of the insemination (Flowers, 1998).  If a sow is in estrus for more than one day, then she will have an increased reproductive performance with multiple inseminations.  These advantages are not seen in sows with an estrous length of less than one day (Flowers and Esbenshade, 1993).   Sows that have a long wean-to-ovulation interval have longer estrous periods (Soede et al., 1994).  Females with a long wean-to-estrus interval also ovulate during a shorter time period after the onset of estrus.  Females with long wean-to-estrus intervals had reduced litter sizes and farrowing rates (Kemp and Soede, 1996). Weitz et al. (1994) found that shortened wean-to-estrus periods resulted in extended estrous lengths using

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transcutaneous ultrasonography in determining the timing of ovulation. This study reported that sows which came into estrus less than four days after weaning were in estrus 72-96 hours and ovulated 48-72 hours after the onset of estrus. In contrast, sows that came into estrus later than 96 hours after weaning only showed estrus for 48 hours and ovulated between 32 and 40 hours after the onset of estrus.

The time of ovulation plays a very important role in the success of the artificial insemination. Waberski et al. (1994) found that the optimal timing for insemination with liquid semen was during the 12 hours before ovulation to 0 hours after when using liquid semen. They observed decreases in fertility after this time interval, presumably due to increased polyspermy. Based on the number of piglets born alive, Nissen et al. (1997) found that the optimal time for insemination was 28 hours before to 4 hours after ovulation with an insemination cell dose of $2x10^9$. The optimal time for insemination regarding pregnancy rates and litter size is 24 hours before to 0 hours after ovulation regardless of wean-to-estrus interval (Kemp and Soede, 1996). The percent of normal embryos increases with inseminations 24 to 0 hours before ovulation with a single insemination of $3x10^9$ spermatozoa (Soede et al., 1995b). Since most farms inseminate for as many days as the sow shows the standing heat reflex, second inseminations can be occurring in late estrus or into metestrus, after ovulation has taken place. Late inseminations or metestrus inseminations decreased litter sizes (Rozeboom et al., 1997). If the second insemination occurs in the first hours after ovulation, fertilization is positively affected (Soede et al., 1995a).

In order for fertilization to take place, the insemination has to provide an ample number of viable sperm. Bracken et al. (2003) studied the effects of a single low dose

insemination compared to multiple inseminations with a high dose of sperm. It was found that the single insemination of $0.5 \times 10$ sperm did not provide satisfactory fertility results when inseminated at 22 hours after the detection of estrus compared to the multiple inseminations of $3 \times 10^9$ sperm at 10 and 22 hours after the detection of estrus. Usually $3 \times 10^9$ sperm per insemination is considered to be a guideline for optimal fertility. Nissen et al. (1997) found comparable fertility results using $2 \times 10^9$ sperm.

## GnRH Agonist Use

Esbenshade et al. (1990) reviewed the actions of GnRH in sows and found that pulsitile GnRH administration produces follicular development and ovulations in weaned sows similar to what naturally occurs in the follicular phase of the estrous cycle. GnRH agonists, such as Triptorelin, bind the gonadotropin receptors and stimulate a similar response to gonadotropins. Pulse frequency of GnRH agonist administration can elicit different endocrine responses. A high pulse frequency of GnRH agonist causes a significant increase in LH compared to FSH. The opposite is true for low pulse frequencies of the agonist, which causes a significant increase in FSH compared to LH (Jayes et al., 1997). Similar results were found for FSH by Picton et al. (1999) along with a decrease in basal plasma estrogen concentrations. Takekida et al. (2000) saw a similar decrease in estrogen concentrations as well as a decrease in progesterone concentrations in the granulosa cells of large follicles from the sow's ovary when using a

GnRH agonist. The use of GnRH agonists in swine is usually a timed-release mechanism that causes an LH surge to follow as well as ovulation.

Artificial insemination has become widely used world wide for use in breeding of sows. Hühn et al. (1996) published a review on the techniques used to control estrus and ovulation as well as the introduction on timed insemination protocols in the East German pig industry. They reported that the use of artificial insemination in East Germany has risen from 10.6% in 1970 to 86.9% in 1989. As artificial insemination use grew in the industry, the use of pharmaceutical methods of synchronizing estrus and ovulation also grew. In the early 1980's the East German pig industry administered eCG (PMSG) to weaned sows to stimulate estrous behavior. When 1000 IU (first litter gilts) or 800 IUs (sows) was given at 24 hours post weaning, greater than 95% of all gilts and sows were in estrus within 4.5 days. Sows were then examined for estrous behavior and bred two times (12 hours apart). As new pharmaceutical methods became available, administering hCG or GnRH approximately 56 hours after estrous stimulation with eCG induced ovulation. This protocol allowed for the use of a timed insemination protocol where the inseminations took place at approximately 24 and 42 hours after hCG or GnRH. Eventually, the GnRH was replaced with a GnRH agonist that resulted in improved synchrony of ovulation.

One of the most widely used protocols for timed insemination in cattle is "Ovsynch". This protocol consists of an injection of GnRH to lutenize the dominant follicle followed 7 days later by an injection of PGF2$\alpha$ to regress the CL. A second injection of GnRH is give 30 to 36 later to synchronize ovulation followed by timed insemination 16 to 20 hours later (Pursley et al., 1995, Pursley et al., 1997). Short term

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use of GnRH agonists can be used in cattle for inducing ovulation.  Chronic use of GnRH

agonists will significantly damper the pulsatile release of LH (D'Occhio et al., 2000).

Santos et al. (2004) subcutaneously implanted a time-released GnRH agonist, desorelin,

in place of the second GnRH injection in the ovsynch protocol in dairy cattle.  The

agonist was administered in two concentrations of 450μg and 750μg.  These researchers

found that the desorelin implant was successful in inducing ovulation and formation of a

functional CL.  The pregnancy rates were lowered on day 27 and 41 after the timed

insemination took place for the group of cows that received the 750μg implant compared

to the 450μg implants and the cows that received the ovsynch protocol's second GnRH

injection.  Embryonic loss was also decreased for the cows that received the 450μg

implant.

Meyers et al., (1997) used a slow-release implant containing deslorelin acetate, a

potent GnRH agonist to induce ovulation in cycling mares.  The time of ovulation was

reduced from 84.2 hours to 50.2 hours after estrus with the use of the implant.  The

agonist also increased the percentage of mares that were ovulating from 37.7% to 86.1%.

Wenzel et al. (2002) studied the effects of desorelin administered intramuscularly in a

Pluronic® F127 gel formation in place of the second injection of GnRH in the ovsynch

protocol.   The results of this study indicate that deslorelin induces ovulation while the

gel formulations reduced the rate of degradation of the drug and increased the time to

peak LH concentrations during the LH surge.

## General Conclusions

Timing of ovulation, insemination technique, semen handling, stress from breeding and body condition are a few of the factors that affect the reproductive performance of a sow. In addition, a farm must also consider the cost of labor associated with semen collection, heat detection and breeding before using artificial insemination. As the use of artificial insemination as a breeding technique increases, it becomes increasingly important to properly time inseminations in order to maintain a high reproductive performance while reducing labor costs on the farm. It may become even more cost effective to use hormonal biotechnical methods to induce ovulation and inseminate based on a timed insemination protocol. More research is needed however to come up with a cost effective timed insemination protocol.

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**OVULATORY AND REPRODUCTIVE CHARACTERISTICS OF SOWS**

**TREATED WITH AN INTRAVAGINAL GNRH AGONIST GEL**

## Introduction

Artificial insemination is widely used in the United States. Sows are typically checked for estrus one to two times per day and inseminated at least once each day of estrus. Detection of estrus and insemination is one of the greatest expenses on the farm. Moreover, most of the problems associated with artificial insemination are inaccurate detection of estrus and improper timing of inseminations (Flowers and Esbenshade, 1993). The ability to synchronize estrus and ovulation in the sows would allow for the development of timed artificial insemination protocols. These, in turn should decrease the labor and errors involved with artificial insemination. Thus, the objective of this study was to examine the effect of intravaginal GnRH administration on estrus, ovulation, LH, estradiol and cortisol concentrations in weaned sows. This study also examined the efficacy of intravaginal GnRH administration for use in timed insemination protocols.

## Materials and Methods

*Animals*

     This study used seventy-one crossbred sows with an average parity of 3.14 consisting of Landrace, Large White and Yorkshire breeds that were expected to exhibit 100% heterosis.  The parity of the sows ranged from one to ten.  (All sows used tested negative for P.R.R.S., mycoplasma hyopneumonia, parvovirus, leptospirosis, hemophilus parasuis, steptococcus suis, swine influenza and T.G.E.) The boars used in the study were between 3 and 4 years of age.  They were produced with a breeding scheme consisting of  Duroc, Hampshire, Pietran and Spot breeds that was designed to produce 100% heterosis in the terminal animals. All boars were also negative for P.R.R.S., mycoplasma hyopneumonia, parvovirus, leptospirosis, hemophilus parasuis, streptococcus suis, swine influenza and T.G.E.

*Facilities*

     The study was conducted at the North Carolina State Swine Educational Unit, a farrow-to-finish operation.  Sows were brought into the breeding barn on the day of weaning and placed into 1 m x 2 m breeding crates.  This barn is a

curtain-sided building with a flush, under-slat ventilation system.  Here, sows

were fed 1.8 kg of a corn and soybean meal diet fortified with vitamins and

minerals to meet NRC requirements (NRC, 1988).  Sows were housed here for 10

days then moved into a gestation barn into 1m x 2m gestation crates.  This barn is

also a curtain-sided building with a flush, under-slat ventilation system.  Sows

were housed here until 4 to 7 days prior to their estimated farrowing date.  During

gestation, sows were fed, based on body condition score, between 1.8 and 3.6 kg

of a corn and soybean meal diet fortified with vitamins and minerals to meet NRC

requirements (NRC, 1988).  Sows were then moved into a farrowing barn with a

side-wall baffle ventilation system.  The farrowing crate was a bow-bar crate with

measurements of 1.5 m x 2.5 m.  There was an airplane slat flooring pattern with

cement slats underneath the sow, TriBar®, an expanded metal, behind the sows,

and Tenderfoot®, a plastic-coated wire, in the piglet area.  Two heat lamps were

also placed at varying heights in each crate as additional heat sources for the

piglets.  Here, sows were fed a corn and soybean diet ab libitum two times per

day.  The study was conducted every other week for 10 weeks.

*Treatments*

Following weaning, sows were randomly assigned to one of the six

treatment groups.  The treatment groups were: 100μg of Triptorelin and 1.2 %

saline (n = 11), 1.2% methylcellulose gel (n = 12), 100μg Triptorelin and 0.6 %

methylcellulose (n = 10), 100μg Triptorelin and 0.9 % methylcellulose (n = 10),

100μg Triptorelin and 1.2 % methylcellulose (n = 10), 100μg Triptorelin and 1.5

% methylcellulose (n = 9).  The 100μg Triptorelin with saline were the positive

control group and the sows receiving only the 1.2% methylcellulose gel with no

Triptorelin were the negative control group. A plastic artificial insemination

catheter with a slight upward turn on the end was attached to a 15ml syringe with

a short piece of rubber tubing and used to administer the gel into the anterior

portion of the vagina.  This location supports maximal absorption of the

Triptorelin from the gel.  The catheter was placed in the vulva at a 45° angle and

gently pushed cranially through the vagina. Once the catheter reached the cervix,

indicated by the resistance of cervical ridges on the tip of the catheter, the catheter

was pulled caudally about 4 cm.  The gel was then depositied by depressing the

plunger on the syringe.  Signs of bleeding and sow discomfort were recorded.

*Experimental Procedures*

A non-surgical procedure was used to insert jugular cannulas 64 – 72

hours prior to treatment.  Each sow was snared with a rope.  The right side of the

sow's neck was scrubbed twice with a 1.0% iodine soap scrub followed by

application of 70% isopropyl alcohol.  A number 22 scalpel blade was used to

make a small puncture in the jugular furrow.  A 3.5", 13 gauge, thin-walled,

stainless steel needle with a 45º bevel was inserted into the puncture hole and into

the right jugular vein.  Tygon® tubing (I.D.=.04";O.D.=.07"; Fisher Scientific,

Atlanta, GA) was inserted into the needle and into the vein.  About three feet of

tubing was used for each sow of which about 12 inches was placed in the vein.

The tube was flushed with .12M sodium citrate and an 18-gauge blunt was

inserted into the end of the catheter.  The blunt was capped using the tip of a 1 mL

plastic syringe that was cut and crushed at about 0.2 mL down the syringe.  Two

Foam Sealer® (.25" x2" x 2"; Lowe's Home Improvement, Wilkesboro, NC)

patches were used to secure the catheter to the sow's neck with Tag Cement®

(Nasco, Fort Atkinson, WI).  The free end of the catheter was placed in a denim

pouch and secured to the top of the sow's neck with Elasticon® tape (3.5" width;

Carolina Surgical Supply, Raleigh, NC).  All equipment was sterilized using

Nolvasan® (Nasco, Fort Atkinson, WI) before, during and after each procedure.

If necessary, the sows were recannulated using the left side of the neck at 24

hours prior to treatment.

Blood samples were taken every six hours beginning 42 hours prior to

treatment; every two hours from the administration of the treatment for the next

12 hours; and finally every 6 hours for the next 18 hours (32 hours after

treatment).  Figure 1 contains a diagram of the experimental time line.  A total of

about 10mL of blood was collected at each time point. The blood was collected

into two 5mL slip tip sterile syringes (Fisher Scientific, Atlanta, GA).  The first

2mL of blood were discarded.  The next 6ml of blood were collected and placed

in glass tubes (16mm x 100mm; Fisher Scientific, Atlanta, GA).  An addition 1 ml

of blood was collected in a seperate glass tube containing 9 mL of histological

grade acetone (Fisher Scientific, Atlanta, GA).  After collection, the catheter was

flushed with 3 mL of a 0.12 M sodium citrate.  Blood samples were stored in a

cooler at about 4°C for 24 hours, then centrifuged  at 2700 x g at 4°C  to separate

the sera.  The serum was then divided into two separate aliquots and stored at -
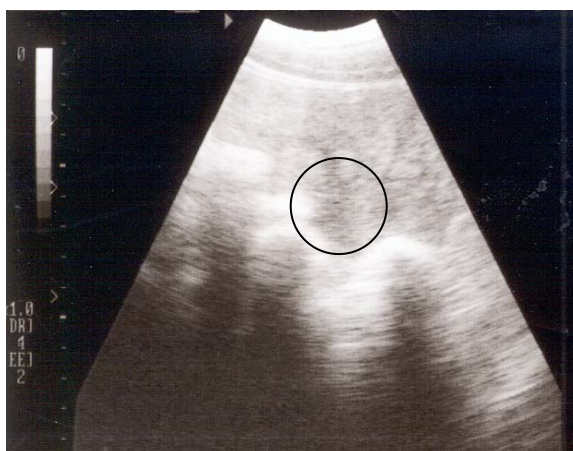
20ºC until analyzed. The acetone samples were immediately stored at -20ºC and shipped to Pennsylvania State University at the conclusion of the study for GnRH agonist analysis.

Sows were checked for estrus every 6 hours beginning 36 hours prior to treatment until 96 hours after treatment (Figure 1). A boar was placed in the walkway in front of 4 - 6 sows. A back-pressure test was performed on each sow to determine if an immobilization response would occur. Sows that became immobile in the presence of the boar for two consecutive checks were considered to be in estrus. Sows were determined to be out of estrus if the immobilization response was not observed for two consecutive detection periods.

Transabdominal real-time ultrasonography was used to determine the time of ovulation. Ultrasound diagnosis took place every 12 hours from 12 hours before administration of treatment until 12 hours after treatment, then every 4 hours until ovulation was confirmed (Figure 2). A 5.0 MHz linear convex probe on a Sonovet 600 (Medison America, Inc., Cypress, CA) ultrasound machine was used to examine the ovaries. Ultrasound transmission gel (Eco-gel; TheraQuip, Greensboro, NC) was applied to the end of the probe and the probe was then placed on the sow's right flank, about 7cm above the last two pairs of nipples. The bladder was found as a reference point and then the probe was rotated cranially until at least one of the ovaries containing follicles, appearing as large dark circles surrounded by a gray circle, was observed. The criterion for ovulation was the subsequent disappearance of these follicles for at least two consecutive examinations (Figure 2).

Small fluid filled follicles can be seen on the sow's ovary.



The fluid filled, dark circles are absent. The sow would be classified as ovulated.

Figure 1.  Ultrasound pictures depicting an ovary that contains follicles and therefore has not ovulated as well as an ovary that does not contain follicles.

Control sows were bred once each day of estrus beginning on the first day of estrus.  Treatment groups were bred on a timed insemination protocol consisting of two matings, 8 and 32 hours after the administration of the treatments.  All sows were bred using mixed semen from two boars that was extended using BTS extender (Minitube of America Inc., Verona, WI).  The

insemination dose consisted of 3 billion spermatozoa.  The semen was stored for less than 48 hours prior to use.  Inseminations were performed by three experienced technicians using a yellow, foam-tip artificial insemination catheter.  The vulvas were wiped with a paper towel prior to inserting the catheter.  The tip of the catheter was lubricated with a nonspermicidal KY Jelly.  The catheter was inserted into the vagina at a 45º angle and then into the cervix using a counterclockwise rotation.  The catheter was then checked for proper placement by gently pulling back on the catheter to make sure that it is locked into the cervix.  The cochette of extended semen was then attached to the end of the catheter and inverted allowing the sow to accept the semen via uterine contractions.

Detection of pregnancy was performed twice.  On day 21 after breeding, the sows were checked for estrus using fence-line boar exposure.  On day 40, pregnancy detection was also performed using real-time ultrasonography.  Fertility data including farrowing rate, number of pigs born alive, percent mummies and stillborns, and lactation length was collected for each sow.
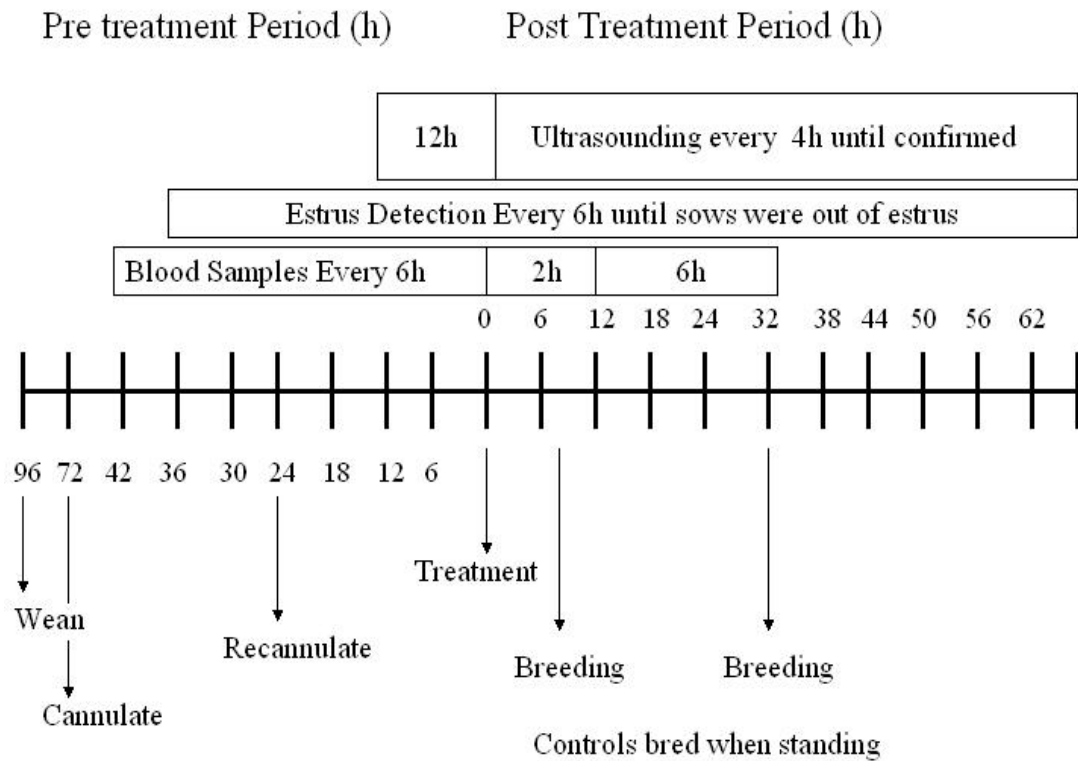
Figure 2. Time line of experimental procedures prior to and following administration of treatment.

*Radioimmunoassays*

Luteinizing hormone concentrations were determined using a validated, double antibody radioimmunoassay at the Rhodes Animal Science Complex at the University of Georgia by Dr. George Rampacek (Kraeling et al., 2000). Estradiol-17β and cortisol concentrations were analyzed using using commercially available radioimmunoassay kits (DPC, Inc; New York, NY) and validated for porcine serum at North Carolina State University (estradiol-17β: Howard and Britt, 1990; cortisol: Metcalf, 1994). The intra- and interassay coefficients of variation for estradiol and cortisol were 4.5% and 6.7%; and 5.2% and 6.1% respectively.

*Statistics*

Statistical analyses were run using Statistical Analysis Software (SAS) version 8.2 (Cary, N.C.). See appendix A.

LH Surge

The duration, peak and amplitude of the LH surge were calculated. The mean concentration of LH was determined for each sow. The standard deviation was then determined for each sow. The LH profile was examined individually at each sampling time beginning at 42 hours prior to treatment. When the concentration of LH was two standard deviations above the mean LH concentration, the sow was then classified as a candidate for the beginning of the LH surge. The LH surge was considered to have begun if two subsequent LH

concentrations were above or equal to this tentative surge value. Therefore the

onset of the LH surge was classified as the time of the last baseline LH

concentration before the rise in concentration. The ending of the LH surge was the

time at which the concentration of LH was back within two standard deviations

from the mean LH concentration, or back to baseline concentrations. The peak LH

concentration was determined as the highest LH concentration during the LH

surge. The amplitude of the LH surge was determined by calculating the

difference in the concentration at the start of the LH surge (the original baseline

value) and the peak value of LH. The duration of the LH surge was the time from

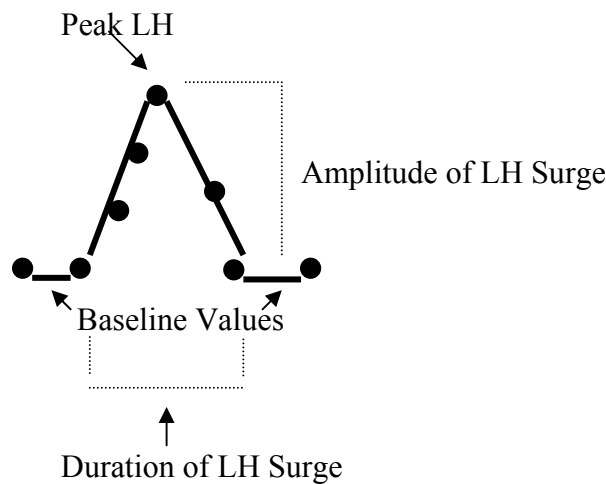the original baseline value to the return to baseline concentration (Figure 3).

Figure 3. Graphical depiction of the LH surge characteristics.

LH surge data was analyzed using the analysis of variance procedures for

general linear models (Snedecor and Cochran, 1989). The model included group

(week 1 through 5), treatment (1 through 6), parity group (group1 = parity 1 and

2, group 2 = parity 3 and greater) and treatment by parity group interaction. Lactation length and previous number of pigs weaned were used as covariates.

Mean LH concentrations were also analyzed over time using analysis of variance procedures for repeated measures (Gill and Hafs, 1971). The model included group (week 1 through 5), treatment (1 through 6), parity group (group 1 = parity 1 and 2, group 2 = parity 3 and greater),treatment by parity group interaction, time, time by parity group interaction, time by treatment interaction, and time by parity group by treatment interaction. Sow nested within treatment and sow nested within parity group were used as error terms to determine treatment effects, parity group effects, and interactions. Lactation length and previous number of pigs born alive were used as covariates.

Esradiol 17-β and Cortisol

The estradiol concentrations and cortisol concentrations were analyzed using analysis of variance for repeated measures (Gill and Hafs, 1971) where time = 0 was the time that treatment was administered. The model included group (week 1 through 5), treatment (1 through 6), parity group (group 1 = parity 1 and 2, group 2 = parity 3 and greater), treatment by parity group interaction, time, time by parity group interaction, time by treatment interaction, and time by treatment by parity group interaction. Lactation length and previous number of pigs weaned were used as covariates. Sow nested within treatment group and sow nested in parity group were used as error terms to test for treatment effects and parity group effects respectively. Estradiol concentrations were also analyzed

using the same model, except the onset of estrus was used as the reference point (time = 0 was the onset of estrus).

## Time of Ovulation and Estrus Length

The time of ovulation and the length of estrus were analyzed for homogeneity of variance using Levene's test using univariate procedures in SAS (Schlotzhauer and Little, 1997). Estrous length and time of ovulation exhibited homogeneity of variance (p=0.72 and p=0.08 respectively) so analysis of variance procedures for general linear models were used (Snedecor and Cochran, 1989). The model included group (week 1 through 5), treatment (1 through 6), parity group (group1 = parity 1 and 2, group 2 = parity 3 and greater) and treatment by parity group interaction. Lactation length and previous number of pigs weaned were used as covariates.

## Reproduction

Farrowing rate, number of pigs born alive, number of stillborns per litter and number of mummies per litter were analyzed using analysis of variance procedures for general linear models (Snedecor and Cochran, 1989). The model included group (week 1 through 5), treatment ( 1 through 6), parity group (group 1 = parity 1 and 2, group 2 = parity 3 and greater) and treatment by parity group interaction. Lactation length and previous number of pigs weaned were used as covariates.

Additional analyses were conducted on reproductive performance, LH surge, cortisol and estradiol concentrations using a dummy variable called insemination classification. Each sow fell into one of three insemination classifications. The first group consisted of all of the negative control sows that were given the 1.2% gel treatment and bred on each day of standing heat. The treated sows were divided into two other insemination classification groups. The first group consisted of treated sows that had been detected in standing heat when the first timed insemination took place. The second group consisted of treated sows that were not in heat when the first timed insemination took place. Analysis of variance procedures for general linear models were used to analyze the data (Snedecor and Cochran, 1989). The model consisted of group (week 1 through 5), insemination classification (1 through 3), parity group (group 1 = parity 1 and 2, group 2 = parity 3 and greater), insemination classification by parity group interaction, time, time by parity group interaction, time by treatment interaction, and time by treatment by parity group interaction. Lactation length and previous number of pigs weaned were used as covariates.

Length of estrus and time of ovulation were also analyzed using the insemination classification variable. Length of estrus and time of ovulation were analyzed with Levene's test for homogeneity of variance and expressed heterogeneity of variance, so nonparametric procedures were used.

# **Results**

*LH Surge*

 A complete LH surge did not occur in the sampling period for all sows.

Sows in which a complete LH surge did not occur over the sampling times were

not used in the analysis.  The average concentration of LH was $0.9 \pm 0.03$ ng/mL.

The average duration of the LH surge was $18.6 \pm 1.1$ hours with a peak occurring

at $6.5 \pm 2.0$ hours and an average peak concentration of $3.2 \pm 0.2$ ng/mL.  The

average amplitude of the LH surge was $2.2 \pm 0.3$ ng/mL.  There were no treatment

effects for LH surge peak (p=0.20), duration (p=0.87) or amplitude (p=0.86).

Table 1 summarizes the LH surge data for each treatment group.  When individual

LH concentrations were examined over time, no treatment effects (p=0.53) or

parity group effects (p=0.38) were seen.  Figure 4 shows the average LH

concentrations relative to estrus.  Figures 5 and 6 show the average LH

concentrations for all sows and each treatment group.

*Estradiol 17-β and Cortisol*

 The mean estradiol 17-β concentration for all sows was $54.6 \pm 0.9$ pg/mL

and the mean cortisol concentration for all sows was $2.6 \pm 0.1$ ng/mL.  There were

no treatment effects or parity group effects for estradiol concentrations (p=0.17

and p=0.37 respectively).  There were also no treatment effects or parity group

effects in the cortisol concentrations (p=0.66 and 0.96 respectively).  When

estradiol was analyzed relative to the onset of estrus, no treatment or parity group

effects were seen (p=0.16 and 0.58 respectively). Estradiol concentrations did significantly decrease after the onset of estrus (p<0.0001) and after the administration of the treatment (p<0.0001). Figure 5 shows the cortisol and estradiol concentrations by time for each treatment group. Figure 6 shows the average estradiol concentrations by treatment. Figure 7 shows the average estradiol concentration relative to estrus.

*Estrus Length and Time of Ovulation*

The average length of estrus was 46.7 ± 1.5 hours. The average time at which ovulation occurred was 43.1 ± 1.1 hours after treatment was given. The average time of the onset of estrus was 12.5 ± 1.6 hours after treatment. The estrus length was greater for the treatment group receiving the 1.5% gel with triptorelin than the sows receiving only the 1.2% gel (p=0.0388). The time at which ovulation occurred was not affected by treatment (p=0.9452). The variation around the mean ovulation time did, however, decrease as the concentration of the gel increased. The control sows that received the 1.2% gel with no triptorelin had a range of 54 hours over which ovulation occurred. The sows that received the 1.5% gel with triptorelin had a range of only 16 hours. The ranges of the other treatment groups were 30 hours for the 0.6% gel with triptorelin, 26 hours for the 0.9% gel with triptorelin and 20 hours for the 1.2% gel with triptorelin. The sows receiving the saline vehicle with the triptorelin had a range of 8 hours over which ovulation occurred. The sows receiving only the 1.2% methylcellulose gel had a greater variation around the mean time of ovulation than the groups receiving the Triptorelin or the saline (Figure 8).

*Reproduction*

The overall farrowing rate was 87% with an average number of pigs born alive of 10.8 ± 0.3.  The average number of stillborns and mummies per litter were 1.1 ± 0.1 and 0.3 ± 0.1 respectively.  There were no treatment effects for farrowing rate (p=0.8246), number of stillborns per litter (p=0.5041) or number of mummies per litter (p=0.6417).  There were also no differences between the treatment groups in number of pigs born alive (p=0.1560)  The sows in replicate 4 did have a lower number of pigs born alive than the other weeks (p=0.006).  The reproductive performances of the sows are shown in Table 2.

*Insemination Classification*

When the data were analyzed using the insemination classification, there were no differences between the groups in LH surge duration (p=0.67).  The group of sows that received their first insemination while already in estrus had a higher LH surge peak concentration (p=0.0189).  The amplitude of the LH surge was also higher for the sows that were inseminated while in estrus than the sows that were inseminated while not in heat (p=0.0246).  The length of estrus was also longer for the sows that were in estrus when the first insemination took place than the sows that were not in estrus when the first insemination took place (p=0.007).  The time at which ovulation occurred, however, did not differ between the insemination classifications (p=0.1086).  There were also no differences seen between the insemination classifications in farrowing rate (p=0.4493), number of pigs born alive (p=0.1086), number of stillborns per litter (p=0.8357), or number of mummies per litter (p=0.3423).  The cortisol and estradiol concentrations did

not differ between the insemination classifications (p=0.3740 and p=0.5898 respectively).  (Table 3)

## **Discussion**

This timed insemination protocol using an intravaginal gel containing a GnRH agonist was an effective method of inducing ovulation without adversely affecting reproductive characteristics of the sows. In 1995, Soede et al. reported that the average estrus length was 60±11 hours.  Our observed estrus length of 46.7 hours was less than this estimate.  Soede also reported that ovulation occurred on average at 40 hours after the onset of estrus.  On average, the sows in this study ovulated about 31 hours after the onset of estrus.  There is a lot of variability in these estimates.  It has been reported that the length of time from the LH surge peak to ovulation is between 30 and 48 hours (Foxcroft and Van de Weil, 1982, Soede et al., 1994, Liptrap and Raeside, 1966).  The average peak of the LH surge occurred at 10.84 hours after treatment.  Therefore, the interval from LH peak to ovulation was 32.26 hours.

The decrease in variability in the time at which ovulation occurred with increasing viscosity of the gel is important to note.  This suggests that the viscocity of the gel is an important factor in the administration of the GnRH agonist.  All treatments were able to induce ovulation at the concentration of 100µg of Triptorelin, but the treatments with higher viscosity gels appear to

induce ovulation with a decreased variability.   The saline treatment group has the greatest amount of synchrony of time of ovulation.  The higher viscosity of the gel might slow the release of the agonist allowing the concentrations to remain in the blood circulation for longer periods of time.  This could account for the increase in synchrony of ovulation time.  However, ovulation occurred as early as 20 – 24 hours after treatment in the control, 0.6% gel and 0.9% gel groups of sows and not until 34 and 38 hours after treatment in the 1.2% and 1.5% gel groups.  With such a small number of sows in each treatment group, it is hard to speculate whether ovulation was more synchronized in the higher viscosity gel groups.  The sows that ovulated within 24 hours of treatment may have already begun the process of ovulation, therefore were not affected by the administration of the agonist.  It could be due to random chance that the 1.2% and 1.5% groups did not have any sows that ovulated within 24 hours of treatment.  It appears that there is a trend for an increase in the synchrony of ovulation but a larger number of sows in each treatment group would be needed to make any definitive conclusions.

Using saline as a vehicle for the triptorelin appears to produce the greatest amount of synchrony in the time of ovulation.  This could be due to the fact that the saline does not have a time-release mechanism.  The agonist is therefore absorbed quickly resulting in a synchronized LH surge and subsequent ovulation in all sows.

Intravaginal administration of the methylcellulose gel containing a GnRH agonist was an effective means to induce ovulation.  The vagina is a highly

vascular area and is thought to contain GnRH receptors.  Therefore, intravaginal administration of the GnRH agonist induced an LH surge and subsequently ovulation in all treated sows.

LH concentrations in the sows that were treated with Triptorelin did not differ from the control sows.  Previous studies reported peak LH surge concentrations of 4.2 – 23.1 ng/ml (Van De Weil et al., 1981, Prunier et al., 1987).  In this study, the peak concentrations of LH averaged 3.2 ng/ml.  Prunier (1987) reported the length of the LH surge to be between 13 and 20 hours.  The average duration of the LH surge in this study ranged from 16.5 to 20.4 hours.  There were not any differences in LH surge amplitude between the treatment groups.  This is an important observation and indicates that the naturally occurring LH surge in sows is not different from the sows that were induced to ovulate.  There were only a very small number of sows that exhibited a complete LH surge during the sampling period, therefore, it is difficult to speculate whether or not the GnRH agonist administration synchronized the LH surge.   It does not appear as though the saline vehicle or the gel vehicle affected the LH surge characteristics.  This is an interesting observation because the higher viscosity gels appear to increase the synchrony of ovulation without influencing the LH surge.

Estradiol acts in a positive feedback loop during follicular growth with the hypothalamus to cause the release of GnRH and subsequently more estradiol from the ovary.  Estradiol was measured in this study to determine whether the GnRH agonist would have any adverse effects on the concentrations of estradiol

produced and subsequently the concentrations of LH. The estradiol profiles were similar across all treatment groups. Also, estradiol is the primary hormone responsible for estrous behavior. Dial and Britt (1986) reported that the peak concentration in estradiol is responsible for estrous behavior. The peak estradiol concentration here occurred about 12 hours prior to the onset of estrous behavior. Van de Weil (1981) suggested that a rise in prolactin may also play a role in the onset of estrous behavior. Prolactin was not measured, so we are unsure about its role in the onset of estrus behavior. In the present study, estradiol concentrations were decreasing on the day of estrus while LH concentrations were increasing and hitting a maximum concentration about 4-6 hours after the onset of estrus. The release of estradiol and LH was consistent with previous findings by Van de Weil (1981) and Dial and Britt (1986).

It has been shown that cortisol concentrations can be used as an indicator of a displacement of homeostasis or stress (Becker et al., 1985). In this study, cortisol did not have any apparent trends. The levels did not appear to peak during any particular sampling period or during the administration of treatment. Using the concentration of cortisol as an indicator of stress, it would appear that the sows in this study were either not under any significant amount of stress or all under the same amount of stress. Sows that are not in standing estrus will not allow a boar to mount and mate them. The sow will typically have an increase in vocalizations and not stand to be mated by a boar when she is not in estrus. In this study, some sows were bred when they were not exhibiting behavioral signs of estrus. These sows did not show an increase in their cortisol concentrations at

breeding compared to sows that were exhibiting behavioral signs of estrus when bred. Therefore, these sows were not under an overt level of stress. This is an important observation since timed insemination protocols will likely always include breeding some sows that are not exhibiting the standing reflex.

It has been reported that the optimal time for insemination to take place in a timed insemination protocol is about 24 hours prior to ovulation in order to not negatively affect pregnancy rates and litter sizes (Kemp and Soede, 1996). The sows in this study averaged an ovulation time of 43 hours after treatment was administered. With insemination times of 8 and 32 hours after treatment, the inseminations were occurring at about 35 and 19 hours prior to ovulation. This window includes the optimal time of 24 hours prior to ovulation.

Timed insemination protocols that are not effective would typically show a decrease in pregnancy rates or litter sizes. The pregnancy rate for this study was 93% and the farrowing rate was 91.5%. Here the sows that received the 1.2% gel without triptorelin had a lowered number of pigs born alive. All other treatment groups were between 10.6 and 12.1 pigs born alive. These are high enough to suggest that the timed insemination protocol of inseminating at 8 and 24 hours after treatment with the intravaginal GnRH agonist gel provided the sow with fertile semen at the appropriate time for a successful mating.

One of the objectives of this study was to examine the intravaginal gel as a vehicle for the triptorelin. When the triptorelin was administered in the saline vehicle, the reproductive performance was similar to the sows that received the agonist in the gel vehicle. The only difference seen was in the variability in the

time of ovulation that was greater for the saline treatment group than the gel

treatment groups. This could once again be explained by the timed-release

mechanism of the gel being longer than that of the saline vehicle.

Estrus is the time at which the sow is sexually receptive and will allow the

boar to mount and mate. Using estrus detection to breed sows can result in a

number of sows never being bred due to a lack of an observed behavioral estrus.

This does not always mean that the sow is not in a physical estrus. In using a

timed insemination protocol, such as the one used in this study, all sows will be

induced to ovulate and be bred regardless of whether they are exhibiting

behavioral signs of estrus. Sows that were bred while not in heat exhibited a

shorter estrus length without adversely affecting the sow's reproductive

performance. Sows that were not in heat when bred were able to ovulate and

conceive with similar reproductive performance as sow's that were in heat when

bred.

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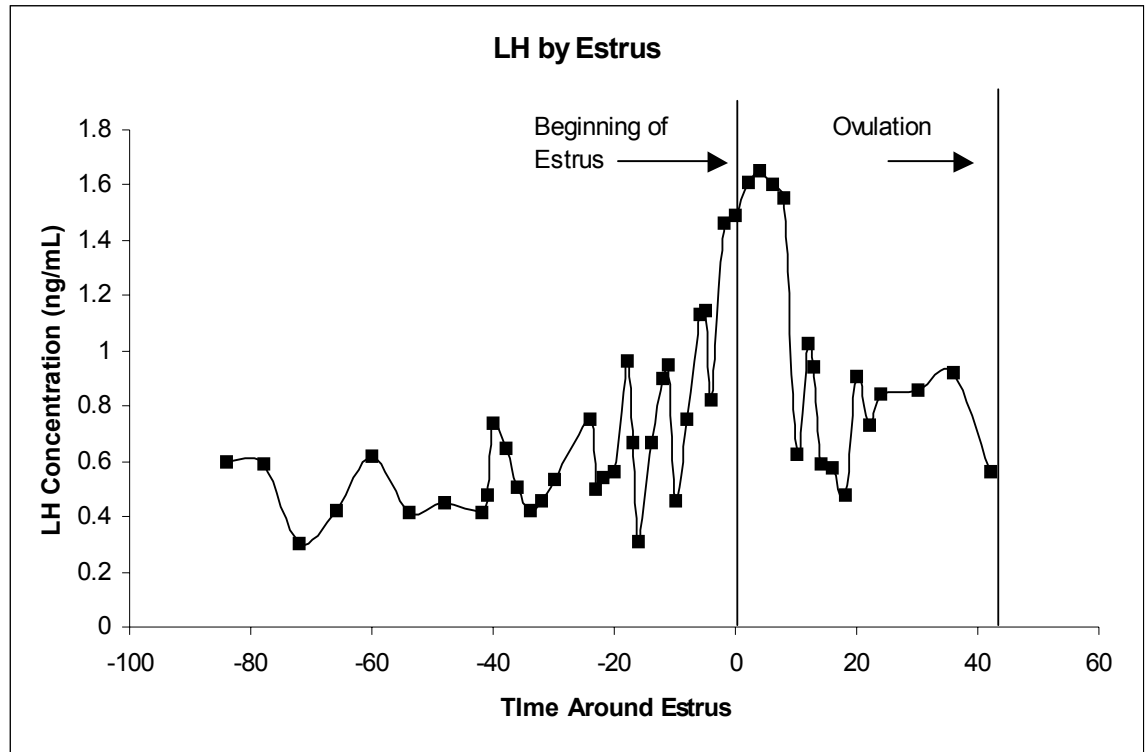
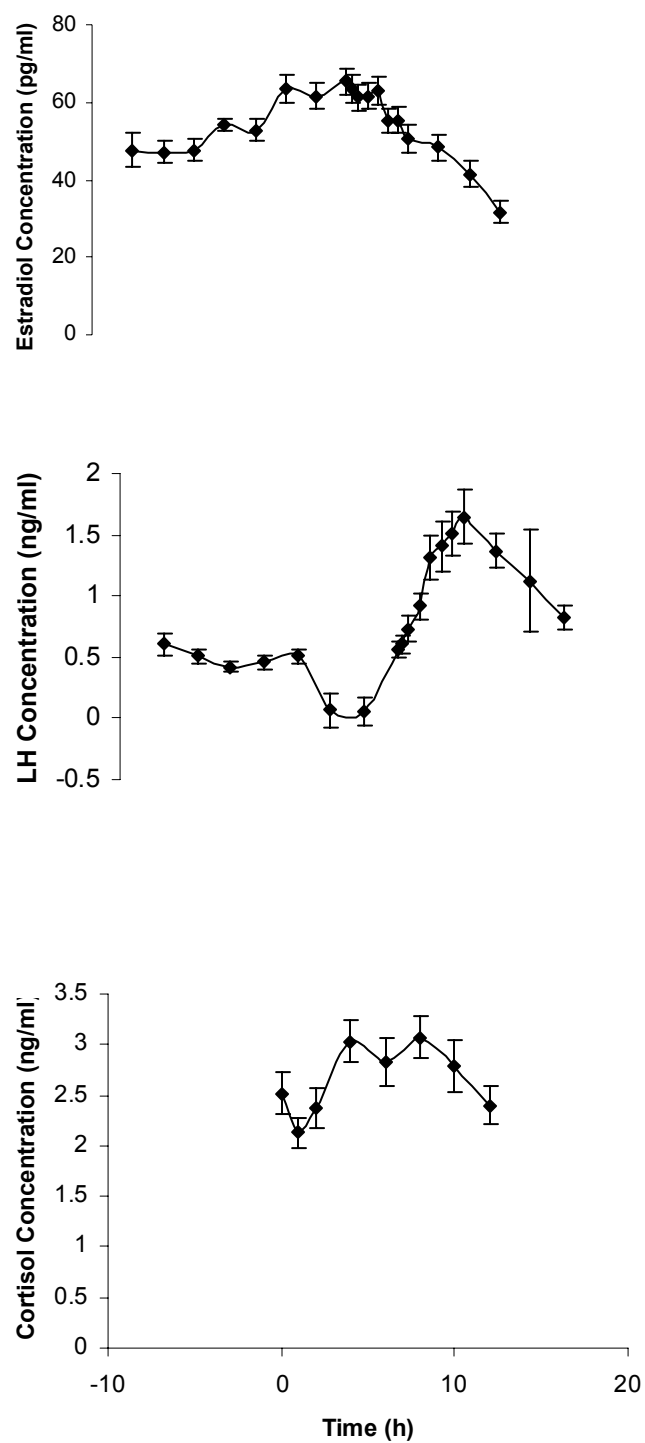Figure 4.  LH concentrations relative to the onset of estrus for all sows.

Figure 5. LH, estradiol and cortisol concentrations (±SEM) (ng/ml) for each sampling time for all treatment groups. Administration of the gel is time 0.
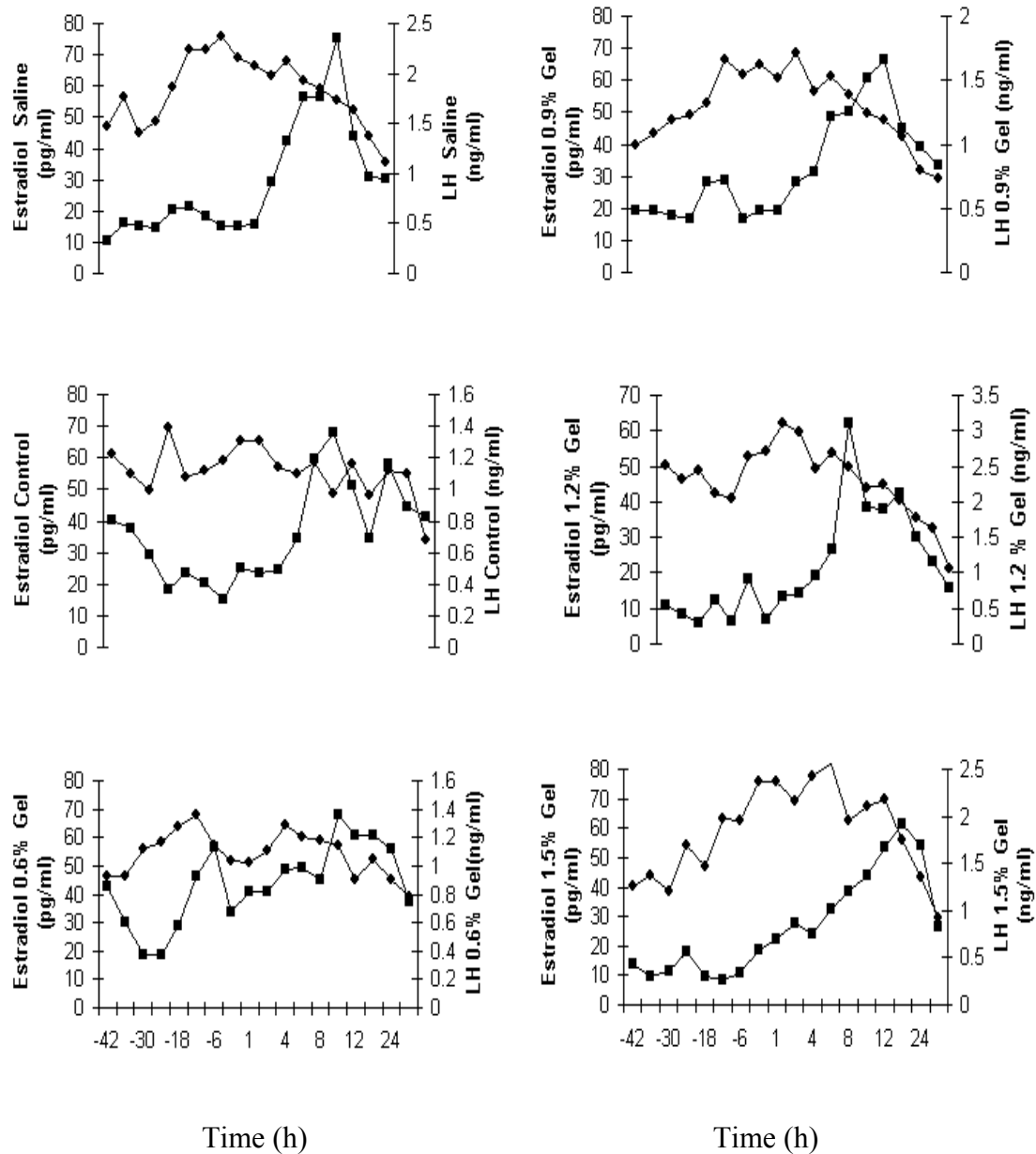
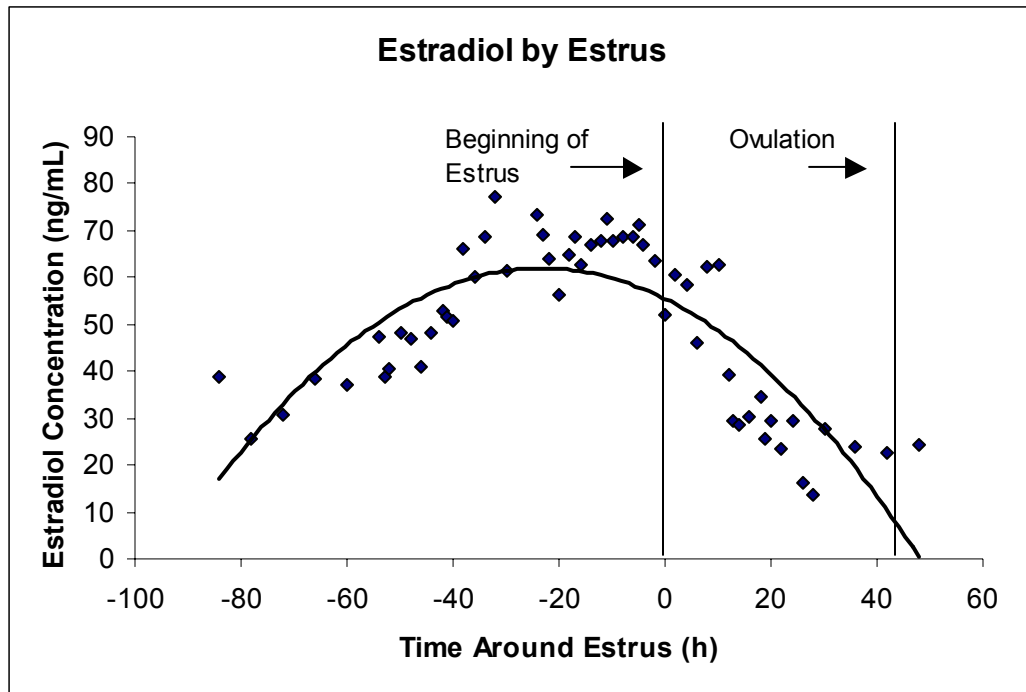Figure 6.  Estradiol 17-β and LH concentrations for each treatment group across sampling times.

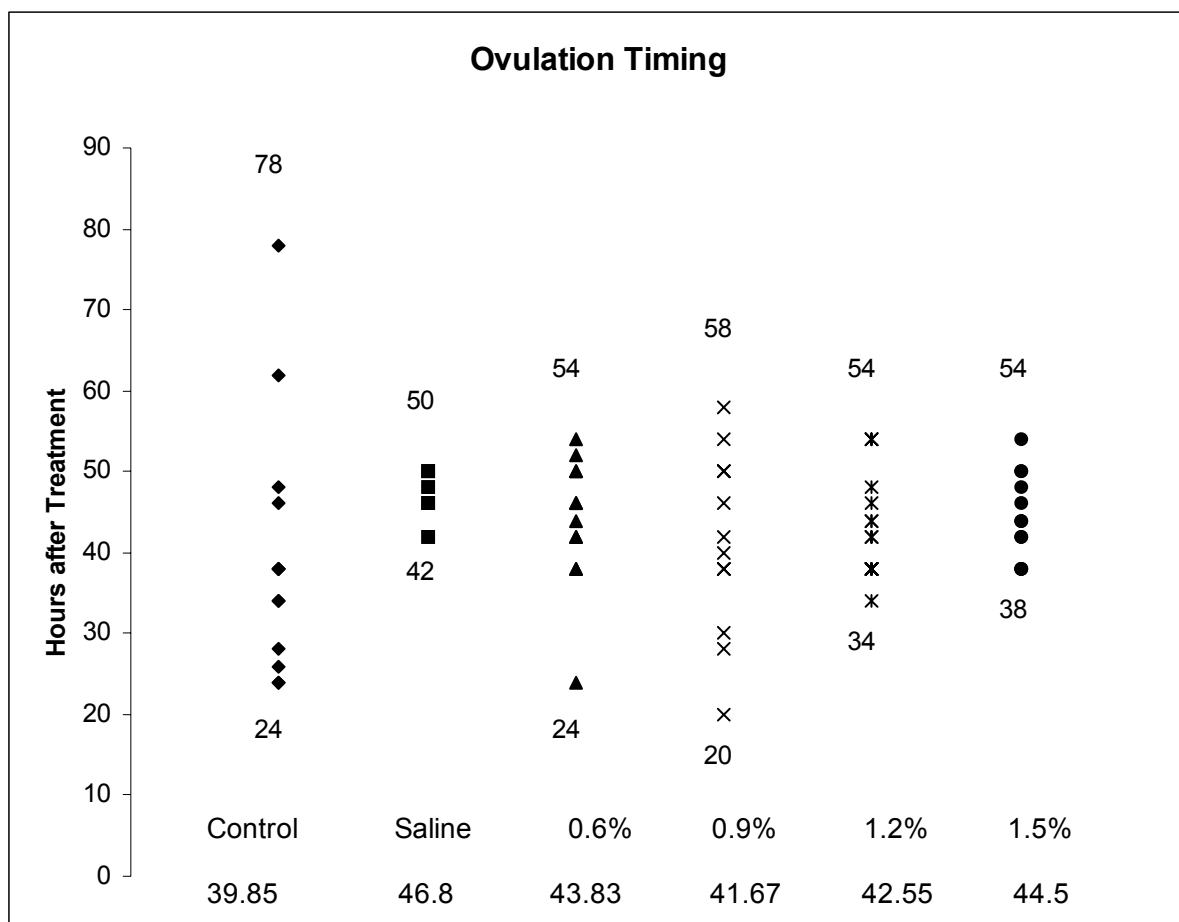Figure 7. Estradiol-17β concentrations relative to the onset of estrus.

Figure 8. Time of ovulation for each sow in each treatment group. Average time of ovulation under each treatment group with the range in values shown for each treatment group.

|  | LH Surge Peak (ng/mL) | LH Surge Duration (h) | LH Surge Amplitude (ng/mL) |
|---|---|---|---|
| Control | 2.4 ± 0.7 | ------ | ------ |
| Saline | 3.9 ± 0.8 | 16.5 ± 1.5 | 3.1 ± 1.1 |
| 0.6% | 3.0 ± 0.5 | 16.9 ± 3.1 | 2.0 ± 0.4 |
| 0.9% | 2.6 ± 0.3 | 19.4 ± 2.2 | 1.7 ± 0.4 |
| 1.2% | 4.2 ± 0.4 | 20.4 ± 1.5 | 2.7 ± 0.5 |
| 1.5% | 3.2 ± 0.5 | 18.7 ± 2.7 | 1.7 ± 0.6 |

Table 1.  LH surge peak, duration and amplitude for each treatment group.

|  | Pregnacy Check 1 (%) | Pregnancy Check 2 (%) | Farrowing Rate (%) | Number Born Alive | Number of Stillborns Per litter | Number of Mummies Per litter |
|---|---|---|---|---|---|---|
| Control (12) | 91.7 ± 8.3 | 91.7 ± 8.3 | 83.3 ± 11.0 | 8.7 ± 1.1 | 1.0 ± 0.4 | 0.2 ± 0.1 |
| Saline (11) | 90.9 ± 8.3 | 100.0 | 100.0 | 12.1 ± 0.8 | 0.4 ± 0.2 | 0.5 ± 0.2 |
| 0.6% (10) | 91.7 ± 8.3 | 91.7 ± 8.3 | 91.7 ± 8.3 | 11.0 ± 1.3 | 1.0 ± 0.4 | 0.3 ± 0.1 |
| 0.9% (10) | 91.7 ± 8.3 | 91.7 ± 8.3 | 83.3±11.2 | 11.6 ± 1.0 | 1.4 ± 0.6 | 0.2 ± 0.1 |
| 1.2% (10) | 91.7 ± 8.3 | 91.7 ± 8.3 | 91.7 ± 8.3 | 11.0 ± 0.5 | 1.4 ± 0.5 | 1.0 ± 0.5 |
| 1.5% (9) | 91.7 ± 8.3 | 91.7 ± 8.3 | 90.1 ± 9.0 | 10.6 ± 1.4 | 1.2 ± 0.4 | 0.4 ± 0.22 |

Table 2.  Reproductive performances for each treatment group.

| Insemination Classification | Control Sows | In estrus at time of first insemination | Not in estrus at time of first insemination |
|---|---|---|---|
| Estradiol (ng/ml) | 55.35±1.35 | 55.08±1.28 | 54.98±2.83 |
| Cortisol (ng/ml) | 2.41±0.13 | 2.76±0.10 | 2.85±0.27 |
| LH (ng/ml) | 0.84±0.05 | 1.00±0.06 | 0.81±0.10 |
| Length of Estrus (h) | 49.50±4.05 | 53.70±2.90 | 44.81±1.72 |
| Time of Ovulation (h post treatment) | 41.17±4.52 | 41.90±1.63 | 44.33±1.36 |
| Farrowing Rate | 1.00±0.00 | 0.79±0.10 | 0.94±0.04 |
| Number of Pigs Born Alive | 9.17±1.01 | 11.53±1.05 | 11.06±0.53 |
| Number of Stillborns per Litter | 0.83±0.32 | 1.13±0.26 | 1.15±0.28 |
| Number of Mummies per Litter | 0.17±0.11 | 0.27±0.12 | 0.62±0.20 |

Table 3.  Reproductive data, estradiol, cortisol and average LH concentrations for all sows by insemination classification.

# Appendix A: SAS Code

*LH Repeated Measures*

```
Proc glm;
Class grp trt time paritygrp;
Model lh = grp trt time paritygrp lactlength prevnow trt*paritygrp id(trt) Test h =
trt   e = id(trt);
Test h = trt*paritygrp  e = id(trt*paritygrp);
Run;
```

*LH Surge*

```
Proc glm;
Class grp trt paritygrp;
Model peak duration amplitude = grp trt paritygrp trt*paritygrp lactlength
prevnow;
Run;id(trt*paritygrp);
```

*Estradiol Repeated Measures*

```
Proc glm;
Class grp trt paritygrp time;
Model estradiol = grp trt time paritygrp paritygrp*trt lactlength prevnow id(trt)
        id(paritygrp*trt);
test h = trt   e = id(trt);
test h = paritygrp*trt  e = id(paritygrp*trt);
means grp time / snk tukey lsd;
run;
```

*Cortisol Repeated Measures*

```
Proc glm;
Class grp trt paritygrp time;
Model cortisol = grp trt time paritygrp paritygrp*trt lactlength prevnow id(trt)
        id(paritygrp*trt);
test h = trt   e = id(trt);
test h = paritygrp*trt  e = id(paritygrp*trt);
```

means grp time / snk tukey lsd;
run;


*Reproductive Data*

Proc glm;
Class grp trt paritygrp;
Model farrow estrusl ovult_t nba stillborns mummies = grp trt paritygrp prevnow
      Lactlength trt*paritygrp;
Run;

*Levene's Test for Homogeniety of Variance*


Proc glm;
Class trt;
Model estrusl ovul_t = trt;
Means trt / hovtest = levene;
Run;