EFFECT OF P.G. 600 ON THE TIMING OF OVULATION IN GILTS TREATED WITH REGU-MATE

by

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ABSTRACT

We previously reported that ovulation rate, but not pregnancy rate or litter size at d 30 post-mating, was enhanced by gonadotropin treatment (P.G. 600; Intervet America Inc., Millsboro, DE) in gilts fed a progestin (Regu-mate; Intervet America Inc.) compared with gilts receiving progestin alone. We hypothesized that P.G. 600 altered the timing of ovulation, therefore mating gilts 12 and 24 h after first detection of estrus, as is common in the swine industry, may not have been the most appropriate breeding regimen. The objective of this study was to determine the effect of P.G. 600 on the timing of ovulation in gilts treated with Regu-mate. Randomly cycling, crossbred gilts (5.5 m of age, 117 kg BW, and 14.7 mm BF) were fed a diet containing Regu-mate to provide 15 mg/d for 18 d. Twenty-four h after Regu-mate withdrawal, gilts received i.m. P.G. 600 (n = 25) or saline (n = 25). Gilts were checked for estrus at 8 h intervals. After first detection of estrus, trans-rectal ultrasonography was performed at 8 h intervals to determine the time of ovulation. Gilts were killed 9 to 11 d after the onset of estrus to determine ovulation rate. All gilts displayed estrus by 7 d after treatment with P.G. 600 or saline. Compared with saline, P.G. 600 increased ($P = 0.07$) ovulation rate (14.8 $\pm$ 1.1 vs. 17.5 $\pm$ 1.0, respectively). The intervals from injection-to-estrus (98.4 $\pm$ 2.7 vs. 110.9 $\pm$ 2.7 h; $P < 0.01$) and injection-to-ovulation (128.6 $\pm$ 2.8 vs. 141.9 $\pm$ 3.2 h; $P < 0.01$) were decreased in gilts treated with P.G. 600 compared with gilts treated with saline. Estrus duration (54.4 $\pm$ 2.3 vs. 53.7 $\pm$ 2.5 h; $P = 0.83$), estrus-to-ovulation (30.2 $\pm$ 2.0 vs. 31.7 $\pm$ 2.2 h; $P = 0.62$) and time of ovulation as a percentage of duration of estrus (55.8 $\pm$ 2.7 vs. 57.5 $\pm$

3.0%; $P = 0.67$) were similar for the P.G. 600 and saline-injected gilts, respectively. In summary, P.G. 600 advanced the onset of estrus and ovulation following termination of Regu-mate treatment and increased ovulation rate. However, treatment of gilts with P.G. 600 had no effect on the timing of ovulation relative to the onset of estrus.

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INTRODUCTION

Many factors influence reproductive efficiency of a swine operation including farrowing rate (number of females farrowing/number of females mated), the number of pigs born alive per litter, and non-productive days (NPDs). Non-productive days accumulate for females that are not pregnant or lactating and include, but are not limited to, the time between a gilt's entry into the "gilt pool" and her first service, and the interval between weaning and first service in sows. According to industry records (Stein, 1992), NPDs, farrowing rate, and pigs born alive per litter were in order, the three most important factors determining if a herd ranked at the top or bottom of a productivity group.

Swine producers typically replace 30% or more of their sows with gilts each year. Since gilts typically have smaller litters and longer weaning to rebreeding intervals than sows, gilts tend to have a negative effect on overall productivity. Furthermore, reproductive efficiency and overall productivity can be negatively influenced if gilts are present within the herd for extended periods of time before first mating, and excessive NPDs accumulate. The ability to synchronize estrus in randomly cycling replacement gilts would decrease NPDs and improve reproductive efficiency. Synchronizing estrus in replacement gilts allows easier entry into sow groups when sows are bred following weaning. Finally, synchronization of estrus allows more efficient use of labor, facilities, and artificial insemination. Treatment of gilts with a combination of an orally active progestin (15 mg Altrenogest [Regu-mate; Intervet America Inc.; Millsboro, DE]/d for 18 d) and gonadotropins (P.G. 600; Intervet America Inc.) administered 24 h after progestin

withdrawal has been successful in synchronizing estrus (Estienne et al., 2001). However, despite increased ovulation rates in gilts treated with altrenogest and P.G. 600, compared with gilts given altrenogest alone, pregnancy rate and number of live embryos at d 30 postmating were similar between groups (Estienne et al., 2001). In swine, maximum fertilization rates occur if females are bred 0 to 24 h before ovulation. Perhaps P.G. 600 alters the timing ovulation in altrenogest-fed gilts, resulting in a needed change in the current regimen of breeding at 12 and 24 h after the first detection of estrus. Real-time ultrasonography allows the accurate determination of time of ovulation in swine.

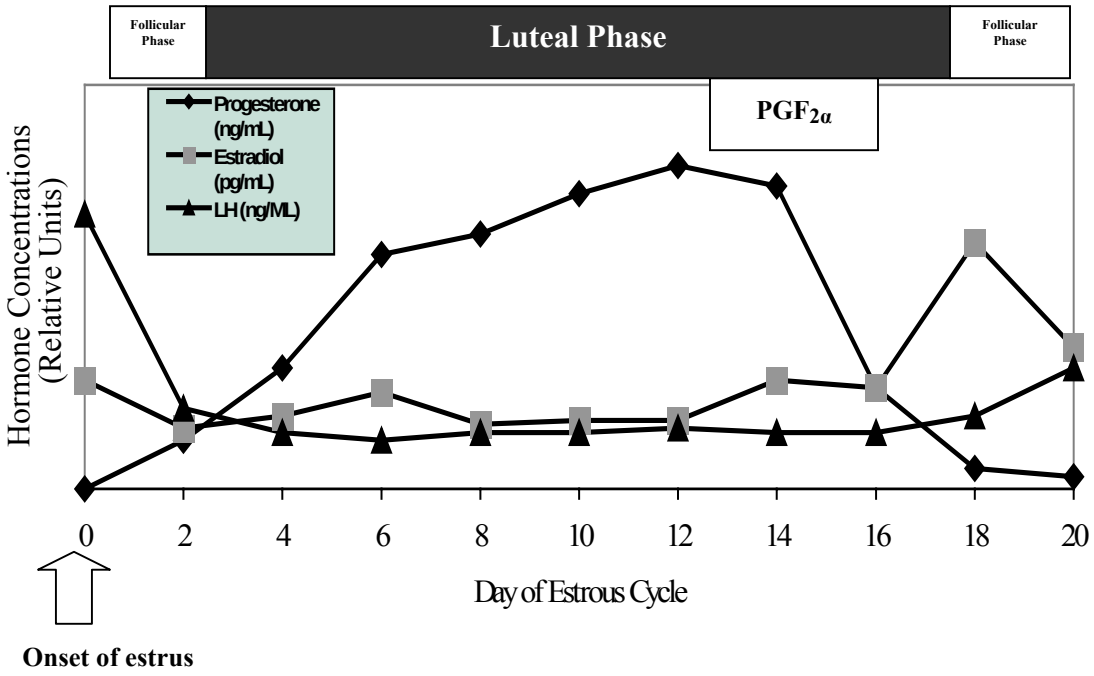
Thus, the objective of this thesis project was to test the hypothesis that P.G. 600 alters the timing of ovulation in altrenogest-fed gilts as determined using real-time ultrasound.

CHAPTER I: REVIEW OF LITERATURE

The Estrous Cycle of the Gilt

General. The porcine estrous cycle is 21 ± 2 d long. Domestic swine are considered polyestrus, meaning gilts generally cycle throughout the year and do not show any evidence of a particular breeding season (Asdell, 1964). This 21-d cycle is divided into a 16-day luteal phase and a 5-day follicular phase. Gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH) are released from the anterior pituitary gland in response to hypothalamic secretion of gonadotropin-releasing hormone (GnRH). During the follicular phase secretion of LH and FSH cause ovarian follicular development, increased estradiol (E_2) secretion, and ultimately, behavioral estrus and ovulation (Kirkwood, 199). A surge of LH (rising in circulating concentration from 1 ng/mL to 8 ng/mL) coinciding with the onset of estrus causes ovulation (Hansel et al., 1973). Throughout the luteal phase, corpora lutea (CL) produce progesterone (P_4) that inhibits GnRH secretion and thus LH and FSH release and thereby prohibits the growth of follicles past the medium-sized stage. As a consequence, onset of estrus does not occur. At approximately d 12 to16 of the luteal phase, prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) is secreted by the uterus causing regression of the CL and termination of luteal production of progesterone. Following regression of the CL, the follicular phase takes place for approximately the next 5 d. Since the P_4 block of the hypothalamic secretion of GnRH is removed, resumption of appropriate secretory patterns of the pituitary gonadotropins takes place leading to follicular growth, estrus and ovulation. Figure 1 shows the patterns of hormone secretion throughout the estrous cycle of a gilt.

Figure 1. Hormone secretion patterns of a cycling gilt.



Estrus/Ovulation. Under the influence of increasing levels of E_2, the follicular phase is characterized by changes in the behavior of the gilt including reduced appetite, mounting of other females, swelling and color changes of the vulva, cloudy mucous discharge from the vulva, and during estrus, the lordosis response. Sexual receptivity generally lasts 2 to 3 days in gilts with a range of 1 to 4 d (Eliasson, 1991).

The timing of ovulation varies but on average occurs about 40 h after the LH surge and the onset of estrus in a gilt when the estrus period lasts approximately 2 d (Niswender et al., 1970). However, when the estrus period lasts greater than 2 d, ovulation occurs at 75 % of the duration of estrus (Niswender et al., 1970). Weitze and Waberski (1996) found the time of ovulation in gilts to vary from 23 to 48 h after the onset of estrus and Martinat-Botte et al. (1995) found the time of ovulation to vary from 26 to 50 h after the onset of estrus. The number of oocytes released during ovulation varies with a range of 10 to 24 depending on age, parity, nutrition, and breed. Typically,

ovulation rate at pubertal estrus is lower than at subsequent estruses. Following ovulation, CL form as solid masses within 6 to 8 d (Warnick et al., 1950).

The time taken to release all oocytes during ovulation varies from 1 to 6 h (Soede and Kemp, 1993). However, Signoret et al. (1972) reported that frequent stimulation by a boar during the onset of estrus may concentrate the ovulation process. Additionally, researchers reported that naturally mated animals tended to ovulate several hours before artificially inseminated (AI) animals due to the presence of E_2 and an unidentified peptide fraction in seminal plasma (Signoret et al., 1972; Weitze and Waberski, 1996).

Following ovulation, rapid proliferation of the granulosa and theca cells lining the ruptured follicle wall takes place. These cells become luteinized as a result of the LH surge, a process that requires the rapid changeover from E_2 to P_4 production and morphological changes into typical steroidogenic cells. The luteinization process proceeds so that a CL has become a solid mass of luteal cells in 6 to 8 days after ovulation with a diameter of 8 to 11 mm. Serum progesterone levels are extremely low (< 2 ng/mL) at the onset of estrus but peak values (> 32 ng/mL) are reached by d 8 to 12 of the estrous cycle (Hansel et al., 1973).

CL Function. Once the LH surge initiates ovulation and the subsequent formation of the CL, continued function of the CL throughout the duration of the estrous cycle is believed to proceed without additional gonadotropic support. Anderson et al. (1967) hypophysectomized sows on d 1 of the estrous cycle and reported that CL were formed and remained active for 15 d. Additionally, Gleeson (1974) suggested that premature failure of the CL early in the estrous cycle was highly unlikely. After injection of PGF 2α into the uterine vein of cyclic sows Gleeson (1974) found that the CL were insensitive to luteolytic factors until they were greater than 10 d old. Gilt plasma P_4 levels typically

reach a peak of 32 ng/mL with a range of 25 to 35 ng/ml from d 8 to 12 of the cycle and remain at these levels until d 14 or 15 when a decline in the P_4 level starts. By the end of the cycle P_4 concentrations return to very low, preovulatory levels.

Estrogens are luteotrophic during the luteal phase of the gilt or sow's estrous cycle. Oral administration or injection of E_2 into a gilt early in the luteal phase is able to prolong the lifespan of porcine CL for several weeks (Dziuk, 1991). After 14 to 15 d of the estrous cycle the lifespan of the CL is determined by the presence or absence of viable embryos. It is necessary for the CL lifespan to be extended if the gilt is pregnant; however, if she is not pregnant the CL will undergo rapid regression and a new wave of preovulatory follicles will develop if the gilt is not pregnant. Morphological regression of the CL and cessation of luteal function mirror one another as plasma P_4 levels in circulation fall rapidly within 1 or 2 d after the onset of regression of the CL in non-pregnant gilts (Dziuk, 1991).

In a pregnant gilt, the CL are maintained as part of the maternal recognition of pregnancy. Initially, the conceptus of the pig produces E_2 11 to 12 d after ovulation that serves as the signal for maternal recognition of pregnancy. Prostaglandin $F_{2\alpha}$ is also produced in significant quantities by the endometrium. However, the E_2 causes the $PGF_{2\alpha}$ to be secreted away from the submucosal capillaries and toward the uterine lumen. Luminal $PGF_{2\alpha}$ has very little access to circulation and thus cannot cause luteolysis. Additionally, there must be at least two conceptuses present in each uterine horn to maintain pregnancy. If two conceptuses are not present in each uterine horn $PGF_{2\alpha}$ will be secreted into the uterine capillaries causing luteolysis (Senger, 1997).

Luteinizing Hormone. In the gilt, the preovulatory LH surge begins to rise in the early hours of estrus, reaching a peak of 8 ng/mL before ovulation or more before

returning to basal levels of 1 to 2 ng/ml about 43 h after ovulation (Brinkley, 1981). The LH surge is triggered by the increasing concentration of E_2 secreted by follicles destined to ovulate during a gilt or sow's estrus period (Soede et al., 1998). The E_2 is believed to act primarily on the central nervous system to cause an LH surge via the stimulation of GnRH secretion (Kraeling and Barb, 1990). The LH surge initiates maturation of the oocyte in the preovulatory follicles and the rupture of the follicle wall. In the ovulatory follicles, the primary oocyte stops its first meiotic division about 20 h after the LH surge and continues with its reduction division, forming its first polar body approximately 2 h before ovulation. Meiotic maturation typically lasts 42 to 44 h in gilts (Hunter, 1966).

The frequency and amplitude of LH pulses vary throughout the estrous cycle. In the mid-luteal phase pulse duration is 60 to 80 min and LH concentrations reach levels similar to those achieved just prior to ovulation. The presence of LH pulses during the mid-luteal phase seems to be associated with the process of follicular maturation or atresia. However, in the late follicular phase there is an absence of pulsatility that continues until the onset of the preovulatory surge of LH. During this phase E_2 concentrations are high suggesting that E_2 suppresses the appearance of LH pulses in the late stages of the cycle. Additionally, it has been shown that exogenous E_2 can inhibit LH secretion in ovariectomized sows and prepubertal gilts. (Gordon, 1997). Pulsatile LH secretion is caused by the pulsatile secretion of GnRH from the hypothalamus. Administration of exogenous GnRH can induce the onset of estrus and ovulation in prepubertal gilts (Lutz et al., 1985).

In contrast, pulsatile release of LH can be stopped by hypophysial stalk transection or by passive immunization against GnRH (Esbenshade et al., 1986). Estienne et al. (1989) showed that P_4 will not suppress the secretion of LH in response to

pulsatile injections of GnRH in a hypophysial-stalk transected pig. This finding suggests that negative feedback action of P_4 on LH secretion occurs at the level of the hypothalamus rather than directly on the pituitary gland. Progesterone secretion from the CL acts on the hypothalamus to suppress GnRH, which in turn causes a decrease in LH production from the anterior pituitary gland. When LH secretion is suppressed, follicular growth and development do not occur (Senger, 1997).

Follicular Dynamics Throughout the luteal phase of the estrous cycle of a gilt, there are 40 to 50 antral follicles present on the ovaries each measuring approximately 3 to 6 mm in diameter; however, about 50 % of these follicles become atretic meaning that the antrum of the follicle disappears and degenerative changes in the antral follicle occur. Guthrie et al. (1995) examined follicular atresia and observed that the follicle population was maintained by a physiological equilibrium between follicle proliferation and apoptotic programmed cell death. During the follicular phase of the estrous cycle, this equilibrium is disrupted when new follicles undergo preovulatory maturation and ovulation. The remaining follicles that do not experience this phenomenon die and are not replaced until after ovulation.

Immediately following ovulation there is a rapid increase in the number of follicles; however, from d 4 until d 20 of the estrous cycle the number of follicles remains the same since there is no evidence of follicular waves in pregnant or non-pregnant gilts or sows. Additionally, no evidence of follicular growth or change in follicle size has been shown until d 15 of the estrous cycle. The non-atretic follicles that measure 3 mm or greater, typically grow at a rate of 1 mm/d from d 15 to 20 of the estrous cycle. Between d 15 and d 20 of the cycle, ovulatory follicles are recruited from a pool of small follicles (Ryan et al., 1994). The recruitment of follicles occurs simultaneously to the

elevation of utero-ovarian vein plasma concentrations of $PGF_{2\alpha}$ that occurs between d 12 and 16 of the estrous cycle, leading to luteolysis (Ratky et al., 1995) Regression of the CL and the related decline in P_4 levels allows for the gonadotropins to cause selection from the pool of small follicles (Huff and Esbenshade, 1992).

Ratky et al. (1995) studied ovarian follicle development in gilts using the endoscopy technique during the first three estrous cycles. They observed that follicles of 4 mm in diameter on d 14 matured to the preovulatory stage, while 2 to 3 mm follicles became atretic. The follicles that matured to the preovulatory stage grew approximately 1 mm/d from d 14 to d 19. Additionally, they found that ovulation rate increased throughout the first three estrous cycles of gilts. The number of ovulations was 8.0, 9.5, and 11.0 through the first, second and third estrus periods, respectively.

In contrast to the work done by Ryan et al. (1994), Guthrie and Cooper (1995) found that the first period of follicular development after ovulation in the gilt results in a tenfold increase in the number of medium-sized (3 to 6 mm diameter) follicles between d 3 and d 8 of the estrous cycle. Additionally, they showed that there was continuous growth and atresia of follicles between d 7 and d 15 of the cycle, however, follicular dominance and waves of follicular growth and atresia did not occur.

Most researchers agree that there is no evidence of follicular dominance or waves of follicular growth and atresia throughout the porcine estrous cycle (Ryan et al., 1994, Ratky et al., 1995, Guthrie and Cooper, 1995, and Guthrie et al., 1995). However, Guthrie and Cooper (1995) believe that there is continuous growth and atresia of follicles throughout the luteal phase until ovulatory follicles are selected between d 14 and 16 of the estrous cycle. In contrast, Ryan et al. (1994) and Ratky et al. (1995) agree that the number and size of follicles remain the same from initial growth immediately after

ovulation to d 14 to16 of the estrous cycle. At this time they believe that follicles 3 mm or greater continue to grow and are eventually selected for ovulation and follicles less than 3 mm in size undergo atresia.

In the follicular phase, the follicles that eventually ovulate grow from 3 to 5 mm in diameter on d 15 to about 7 to 11 mm at the time of ovulation. Similar to LH, plasma FSH concentrations are low during the beginning of the follicular phase in part due to suppression by E_2. Low plasma FSH concentrations occur during the highest and most prolonged period of E_2 secretion. Additionally, follicles secrete inhibin that causes negative feedback on the anterior pituitary gland resulting in decreased FSH secretion. As FSH levels decrease LH levels begin to increase (Senger, 1997). In the follicular phase, the follicles mature, granulosa cells in the follicles proliferate and produce serum levels of E_2 that reach a threshold of 60 to 90 pg/mL on about d 19 of the cycle. This period is followed by the onset of the LH surge and ovulation. Thus, it seems that E_2 initially suppresses FSH and LH levels, however, later it induces the preovulatory surge of LH (Hunter et al., 1972). Following the preovulatory LH surge, E_2 secretion by the follicles declines abruptly (Senger, 1997).

Dial and Britt (1986) suggested that the immediate postovulatory period which results in the abrupt decline in circulating levels of E_2 permits a marked increase in the secretion of FSH that continues for 2 to 3 d after estrus. Additionally, they suggested that the increased release of FSH is probably in response to the disappearance of follicular sources of inhibin, which also has negative feedback effects on the release of FSH.

Real-time ultrasonography swine

Until very recently, real-time ultrasonography was only used in swine as a method of pregnancy detection. However, with the development of higher quality ultrasound

machines and more appropriate transducers, real-time ultrasonography of the ovaries has become more feasible and widely used (Lucy, 1999). A few studies have evaluated follicular growth and development in gilts and sows after weaning. These experiments allow researchers to evaluate the time of ovulation during estrus and consequences of ovulation time relative to insemination time on fertilization rates (Soede and Kemp, 1999). Lucy (1999) conducted a study to evaluate ovaries of sows and gilts using real-time ultrasonography. Table 1 shows the results from that study.

Soede and Kemp (1999) conducted a study evaluating ovarian structures through different stages of the estrous cycle. Follicles appeared on the ultrasound image as small black circles in an otherwise grayish area of ovarian tissue. They found that follicles were too small to count the first 2 d after ovulation. However, from d 3 after ovulation onwards, the follicles were $\geq$ 5 mm in size and CL were present. During the follicular phase, the follicles grew to pre-ovulatory size (6 to 8 mm). Corpora lutea were difficult to distinguish in the ultrasound images, as they appeared grayish in the otherwise grayish area of ovarian tissue. Soede and Kemp (1999) also conducted a study to evaluate follicle size in multiparous sows during the first estrus after weaning using real-time ultrasound. They measured follicle size every 4 h from 20 h after the onset of estrus through ovulation to evaluate follicular growth. Table 2 shows the results from this study.

Real-time ultrasonography has allowed researchers to assess duration of ovulation, influences of seminal plasma on ovulation, the time of ovulation relative to estrus, and consequences of the interval between insemination and ovulation on fertilization rates and embryonic development (Knox and Althouse, 1999). In the swine industry, gilts and sows are normally bred at designated times based on the onset of estrus

Table 1. Ultrasonic evaluation of swine ovaries at different stages of the estrous cycle (Lucy, 1999).

Type of Ovary	Ultrasound Appearance
Type I Anestrus*	Numerous small follicles < 2 mm diameter. Flaccid cervix
Type II Anestrus*	Numerous small follicles 2 to 5 mm diameter. Flaccid cervix.
Estrus	Turgid cervix, follicles 7 to 8 mm diameter, few small follicles
Near Ovulation	Less turgid cervix. Triangular follicles, 7 to 8 mm diameter, few small follicles.
Recently ovulated	Less turgid cervix. Ovary without follicles, may see developing CL.
Ovulated > 3 d ago	CL with some follicles (2 to 3 mm). Flaccid cervix.
Cystic	15 to 30 mm follicles. May have CL present. Flaccid cervix.

*Type I anestrus is defined as sows having primarily 2 mm follicles at the time of weaning and a delay in follicular development after weaning.
* Type II anestrus is defined as sows initiating follicular development after weaning but failing to grow many follicles larger than 5 or 6 mm.

Table 2. Follicle size in multiparous sows during the first estrus after weaning (Soede and Kemp, 1999).

Time of scan	Mean size ± SD (mm)	Range (mm)
24 h before ovulation	6.9 ± 0.8	5.5 – 9
16 h before ovulation	6.9 ± 0.7	5 – 9
8 h before ovulation	7.1 ± 0.8	5 – 10
4 h before ovulation	7.0 ± 0.8	5 – 9
At ovulation	7.0 ± 0.7	4 – 9.5

and the frequency of estrus detection. In addition, some producers inseminate females repeatedly in a timed manner as long as they are in standing estrus. However, recent data

provides evidence that the time of ovulation varies after the onset of estrus (Mburu et al., 1995; Kemp and Soede, 1996; Soede et al., 1995; Nissen et al., 1997; Almeida 2000). Based on this research, improving reproductive performance may be possible by using transrectal real-time ultrasound to evaluate follicular growth and determine time of ovulation (Knox and Althouse, 1999).

Timing of insemination relative to ovulation

The time elapsed from insemination to ovulation influences the rate of fertilization in swine. Ovulation timing in relationship to the onset of estrus is directly related to the duration of estrus. Kemp and Soede (1996) conducted a study investigating the relationship of weaning-to-estrus interval (WEI) to timing of ovulation and fertilization rates in sows. Two experiments utilizing 201 multiparous sows were conducted to characterize this relationship. Experiment 1 related the effects of time of insemination relative to time of ovulation, as determined by ultrasonography, on fertilization rate in sows. Experiment 2 evaluated the importance of a second insemination after ovulation and its effect on fertilization rates. In both experiments, estrus duration averaged 53 ± 1h and ovulation occurred at 37 ± 1 h or 71% of the duration of estrus. Additionally, the duration of estrus was negatively related to WEI (r^2 = 0.23; $P < 0.001$). For sows in estrus on d 3, 4, 5, and 6 after weaning the interval from the onset of estrus to ovulation averaged 41 ± 1, 37 ± 1, 34 ± 1, and 24 ± 4 h, respectively. There were no significant differences ($P = 0.5$) in ovulation rates among sows with varying WEI. Sows inseminated 0 to 24 h before ovulation had a significantly greater percentage (92 %; $P < 0.05$) of normal embryos present at 120 h after ovulation compared to those sows that were inseminated more than 24 h (56.2%) before ovulation or 0 to 16 h (70.2%) after ovulation. These results suggest that negative effects of

increased WEI on subsequent litter size and farrowing rate could be a result of sub-optimal timing of insemination relative to the time of ovulation and not the result of inherently poor fertility in sows that exhibit estrus later after weaning. In commercial operations sows are typically inseminated at a fixed time after the onset of estrus. However, these results indicate that commercial producers may need to alter their breeding regimens according to WEI in order to achieve optimum fertilization rates. Additionally, the authors suggested that the time of ovulation could be estimated from the interval from weaning to onset of estrus since estrus duration was negatively related to WEI.

Nissen et al. (1997) conducted a similar study using real-time ultrasound to evaluate the influence of time of insemination relative to time of ovulation on farrowing frequency and litter size in sows. Crossbred, multiparous sows (n = 143) were weaned and observed for estrus every 8 h until the end of estrus to determine estrus duration. Ultrasound evaluations were conducted every 6 h beginning 12 h after the onset of estrus to determine the timing of ovulation. Sows were inseminated at one of four times: the onset of estrus, 12 h after the onset of estrus, 24 h after the onset of estrus, or 0 to 12 h after the detection of ovulation via ultrasound. Results from this study indicated that the average interval from weaning to the onset of estrus was 92 ± 13 h and the duration of estrus was 60 ± 14 h. Ovulation occurred on average 71 ± 14% of the way through estrus. This percentage decreased slightly but significantly ($P < 0.0003$) with increased duration of estrus. Those sows bred ≤ 24 h before ovulation to ≤ 4 h after ovulation had the highest number of embryos (20.3; $P < 0.05$) recovered at d 10 after ovulation. There were fewer non-pregnant sows (16.3% vs. 62.5%; $P < 0.05$) and an increased total number of piglets born per sow (13.7 vs. 9.0; $P < 0.05$) when insemination occurred from

28 h before to 4 h after ovulation compared to those bred > 28 h before ovulation and < 4 h after ovulation. Therefore, these results, which are similar to the study conducted by Kemp and Soede (1996), indicated that optimal time for insemination was between 28 h before to 4 h after ovulation. More specifically, they identified the optimum time for insemination to be 10 ± 6 h before ovulation.

Soede et al. (1995) conducted a study investigating the effects of time of insemination relative to ovulation on fertilization rate. Crossbred sows (n = 151) were checked for estrus every 8 h beginning 62 h after weaning. At variable times beginning at the onset of estrus, sows were artificially inseminated with sperm cells from a mature boar. Transrectal ultrasound was performed every 4 h beginning 16 h after the onset of estrus to assess timing of ovulation. Sows were killed 106 to 136 h after ovulation to assess ovulation rate, and recovery rate of embryos and oocytes. Fertilization rates for each 8 h insemination to ovulation interval were as follows: > 48 h, 35 % (n = 1); 48 - 40 h, 51 ± 36 % (n = 6); 40 - 32 h, 54 ± 36 % (n = 14); 32 - 24 h, 79 ± 32 % (n = 19); 24 - 16 h; 94 ± 11 % (n = 24); 16 - 8 h 92 ± 21 % (n = 24); 8 - 0 h, 95 ± 22 % (n = 21); 0 to –8 h, 75 ± 38 % (n = 26); –8 to –16, 74 ± 43 % (n = 15); and > -16 h, 0 % (n = 1). For sows that ovulated between d 3 and 7 after weaning the duration of estrus was 50 ± 13 h, onset of estrus to ovulation was 35 ± 8 h, and the ovulation timing relative to the duration of estrus was 72 ± 15 %.

When insemination took place between 0 and 24 h before ovulation, the percentage of normal embryos recovered was significantly greater than when insemination took place > 24 h before ovulation or after ovulation (100 vs. 59%; $P <$ 0.05). From these results the authors concluded that fertilization rate was optimum when insemination occurred 0 to 24 h before ovulation. The authors suggested since low

numbers of accessory sperm cells were present in the embryos, apparently too few sperm were present at the site of fertilization when insemination occurred more than 24 h before ovulation.

Mburu et al. (1995) conducted a study evaluating the efficacy of using transrectal ultrasonography to determine timing of ovulation in multiparous sows compared to using estrus symptoms and hormonal profiles to determine ovulation timing. Multiparous sows (n = 24) in their second or fourth parities were studied during two consecutive estrus periods after weaning. Estrus was monitored every 4 h using the back-pressure test in the presence of a mature boar. Follicular development was tracked in each sow using transrectal ultrasonography. Scanning of the ovarian follicles occurred once a day after the onset of proestrus and twice a day during late proestrus through the onset of estrus and continued twice daily through ovulation and the end of estrus. Proestrus was defined as the period of the estrous cycle between luteolysis and the onset of estrus. Additionally, blood samples were collected starting on day 17 of the first estrous cycle and were collected every 12 h until the onset of estrus, after which the frequency of collection became every 2 h until slaughter after ovulation occurred.

The mean interval from the onset of estrus to ovulation was 37 ± 2.1 h and the mean duration of estrus was 56 ± 7.9 h. Therefore, the time of ovulation as a percentage of estrus was 68 ± 7.7 %. The diameter of the largest follicle at the onset of estrus and at ovulation was 6.3 ± 0.5 mm and 9.3 ± 0.5 mm, respectively. The mean intervals from peak E_2 concentration to the onset of estrus and to ovulation were 1 h and 44 h, respectively, with a range in the interval from peak E_2 concentration to the onset of estrus of –10 h to 22 h. The mean intervals from LH peak concentrations to the onset of estrus and to ovulation were 12 h and 35 h, respectively, with a range in the interval from peak

LH concentrations to the onset of estrus of –10 h to 32 h. Based on the results of this and other studies the authors concluded that repeated ultrasonographic examination of the ovaries during one estrus can be used as a more accurate tool to predict ovulation timing during the subsequent estrus compared to hormone analysis, since such large variations typically occurred between sows when measuring intervals from E_2 and LH peak to onset of estrus and ovulation.

Almeida et al. (2000) evaluated the time of ovulation in relation to estrus duration in gilts. They used 92 terminal line cyclic gilts that were experiencing their third estrous cycle. On d 19 of the third estrous cycle, gilts were exposed to boars every 6 h for detection of estrus. Starting 24 h after the onset of estrus, transcutaneous ultrasonography was performed every 6 h to determine timing of ovulation. On average estrus duration was 52.6 h (range: 30 – 72 h) and ovulation occurred on average 44 h (range: 30 – 60 h) after the onset of estrus. Therefore, ovulation occurred at about 85% of the duration of estrus. Additionally, the time of ovulation during estrus was positively correlated with the duration of estrus so that when the duration of estrus was increased, the timing of ovulation relative to the onset of estrus increased ($r^2 = 0.57$, $P = 0.0001$). Studies previously mentioned (Mburu et al., 1995; Kemp and Soede, 1996; Soede et al., 1995; Nissen, 1997) in which ultrasonography was used to determine ovulation timing in sows reported that ovulation occurred at approximately 70% of the duration of estrus period. However, these authors suggest (Almeida et al., 2000) that gilts ovulate later in estrus than sows at about 85% of the duration of estrus. Therefore, different breeding strategies may need to be employed for gilts and sows.

Theoretically, the optimal time fertilization rates depend on the frequency and accuracy of estrus detection since producers inseminate gilts or sows relative to the onset

of estrus. In the study conducted by Almeida et al. (2000), estrus was detected every 6 h. However, most commercial farms check for estrus every 12 h or every 24 h. Thus the accuracy of both duration of detecting standing estrus and time of ovulation would be increased in this study in relation to commercial farms. When estrus is checked every 12 h, the time of onset of estrus provides a less accurate prediction of when ovulation would occur, so fertilization rates would be slightly decreased. These authors hypothesized (Almeida et al., 2000) that when estrus detection is performed at 12 h intervals, the first insemination needs to be done 24 h after the onset of estrus and the second insemination should be done 12 h later. Additionally, they suggested that a third insemination could be justified for gilts that remain in estrus 12 h after the second insemination.

Soede et al. (1998) used ultrasonography to evaluate the process of ovulation in sows. They attempted to relate follicle size during the late follicular phase, the timing of the onset of estrus, ovulation time and subsequent luteal and embryo development. In weaned, multiparous sows (n = 121) pre-ovulatory follicle size was assessed every 4 h starting 20 h after the onset of estrus. Average follicle size at 20 h after the onset of estrus was 6.7 ± 0.8 mm. Follicle size at ovulation, which occurred on average of 35 ± 8 h after the onset of estrus, was 7.1 ± 0.9 mm. Therefore, follicle growth was minimal during the last 15 to 16 hours of estrus before ovulation. Additionally, they demonstrated that the majority of the follicles < 7 mm at 16 h before ovulation increased in size up to ovulation, whereas follicles > 7 mm at 16 h before ovulation did not increase or even decreased in size. The authors suggested that smaller follicles (< 7 mm) before ovulation may undergo compensatory follicular growth just prior to ovulation. In contrast, they suggested that the follicles that remained the same size or decreased in size prior to ovulation may have lost their turgidity, or spherical shape, during the last scanning before

or during ovulation. In 13 sows that were ovulating spontaneously, the deviation between the maximum follicle count and the CL count at 100 h after ovulation when gilts were slaughtered was 0 in 54% of the ovaries (14 of 26), 1 in 27%, 2 in 15%, and > 2 in 4% of the 26 ovaries scanned. In conclusion, the authors (Soede et al., 1998) noted that it is not possible to accurately predict the time of ovulation by just assessing follicular size and growth. Furthermore, they noted that even prediction of the time of onset of estrus based on follicle size during the follicular phase cannot be reliable. However, these authors did offer evidence to support the use of ultrasound as a reliable and accurate tool to study ovulation and follicular development in pigs.

Estrous Synchronization Methods

Altrenogest/ Allyl Trenbolone/ Regu-mate

Researchers intensively searched for a method for over 30 years before a technology was developed that would successfully synchronize estrus in gilts without negatively affecting fertility and embryo quality (Webel, 1978). Initial researchers tested the ability of progesterone and orally-active synthetic progestins to synchronize estrus. The goal of the researchers was to use orally-active progestins to suppress the release of pituitary gonadotropins for 15 to 20 days resulting in inhibited follicle growth and subsequent ovulation. While the pituitary was suppressed, CL on the ovaries regressed spontaneously. Following withdrawal of the progestin treatment resumption of gonadotropin secretion led to a synchronized estrus and ovulation (Webel, 1978). However, these compounds were not totally effective as subsequent estrus was often accompanied by a decline in fertility, imprecise synchronization of estrus, and the development of cystic follicles (Webel, 1978).

An effective method to synchronize the timing of estrus and ovulation in gilts without decreasing fertility was discovered in the late-1970's. The product administered was called altrenogest (otherwise known as 17α-allyl-estratriene-4-9-11, 17β-OL-3-one, allyl trenbolone, A-35957, RU-2267, or Regu-mate [Intervet America Inc., Millsboro, DE]). Preliminary data from Roussel-UCLAF (Paris, France) showed that the new orally active progestin could be fed at doses higher than 5 mg/hd/d for 14 to 18 d to suppress estrus. This protocol resulted in inhibition of follicular growth and ovulation throughout administration. Upon withdrawal of the progestin, estrus was precisely synchronized without development of ovarian cysts or decreased fertility (Kraeling et al, 1981).

The rationale of feeding this orally active progestin to randomly cycling gilts for 14 or more d is that it mimics the biological activity of P_4 and blocks secretion of gonadotropins (FSH and LH) from the pituitary gland. Blocking gonadotropin secretion blocks follicular growth and development which in turn prevents estrus and ovulation from occurring. The progestin does not prevent normal luteolysis, therefore CL regression occurs, but the progestin continues to prevent estrus even after luteolysis occurs. Upon termination of progestin feeding, increased gonadotropin secretion occurs, follicular growth and development begins, and within 4 to 7 d estrus and ovulation occurs (Kirkwood, 1999).

Until recently, altrenogest was only approved for use in horses under the trade name Regu-mate (Intervet America Inc., Millsboro, DE). In the fall of 2003, (Federal Register, October 31, 2003) the United States Food and Drug Administration approved altrenogest for estrous cycle control in sexually mature gilts that have experienced at least one estrous cycle. This product is currently available to producers under the trade name

"Matrix " (Intervet America Inc., Millsboro, DE). Based on the product label, Matrix (6.8 mL containing 15 mg altrenogest/gilt) is fed once daily for 14 d as a top-dressing on a portion of the gilt's daily feed. A fertile estrus can be expected in 4 to 7 d after discontinuation of Matrix feeding

Redmer and Day (1981) conducted a study evaluating ovarian activity and hormonal patterns in gilts fed either 2.5 or 15 mg of altrenogest daily. Progesterone and LH levels remained low in both treatment groups (< 1 ng/mL and < 1.5 ng/mL, respectively) throughout progestin treatment. Plasma estradiol levels were higher throughout treatment for the gilts fed 2.5 mg compared to those fed 15 mg (7 to 13 pg/mL vs. 2.5 pg/mL; $P < 0.01$). A preovulatory surge of LH (4 ng/mL) occurred on the first day of the first post-treatment estrus in the gilts that received 15 mg of altrenogest/d. Gilts receiving 2.5 mg of altrenogest had an increased number of ovulatory sized follicles present during treatment ($P < 0.01$) and a higher frequency of cystic follicles present after withdrawal of feeding compared to the gilts fed 15 mg of altrenogest/d. Consequently, feeding 15 mg of altrenogest was deemed to be more successful at synchronizing estrus in gilts. Feeding 2.5 mg resulted in an increased incidence of cystic follicles and anestrus (11.1% vs. 58.3% for 15 mg and 2.5-mg treated gilts, respectively; $P < 0.05$)

Stevenson and Davis (1982) conducted a study evaluating estrous synchronization and fertility in gilts after feeding altrenogest for 14- or 18- d beginning at estrus or diestrus (part of the luteal phase when there is sustained secretion of P_4). In this study, 160 gilts ranging from 6 to 11 mo of age were used in 4 trials. All gilts were randomly assigned by age and breed to one of four treatment groups in a 2 x 2 factorial design. The main effects were duration of altrenogest feeding (14 vs. 18 d; 15 mg/d) and stage of the

estrous cycle (d 0 = estrus or -1, +1, +2 relative to estrus vs. diestrus +3 to +21 relative to estrus) at treatment onset. Gilts were monitored for estrus twice daily beginning 3 d after the last feeding of altrenogest and gilts were bred 12 and 24 h after the first detected post-treatment estrus. Blood samples were collected on the first and last day of altrenogest feeding. Additionally, blood was collected from 13 control gilts on d 14, 15, 16, and 17 of the estrous cycle and from 15 gilts on the same cycle days during progestin feeding to evaluate the effects of treatment on serum progesterone concentrations during luteolysis. Results from this study indicated that estrus was successfully synchronized in 133 gilts (84.1%) that showed estrus on d 3, 4 or 5 after withdrawal of treatment. Duration of progestin treatment influenced the distribution of estrus after treatment. More gilts in the 18-d treatment group were in estrus on the "peak" day (d 5 after last feeding of altrenogest). More 14-d treated gilts had progesterone concentrations of > 2 ng/mL at the end of treatment ($P < 0.01$). However, the mean interval to estrus was not affected by treatment duration of 14 or 18 d (5.4 vs. 5.3 d, respectively). Furthermore, by 6 d post-treatment, 89.0% and 96.0 % ($P > 0.05$) of the gilts had expressed estrus in the 14- and 18-d treatment groups, respectively. Even though the gilts treated with altrenogest for 18 d showed more precise synchronization of estrus, by d 10 post-treatment 98.0% of the gilts treated for 14 d showed estrus.

Stage of the estrous cycle had a significant effect on synchronization precision. The average interval to estrus was longer ($P <0.01$) for gilts treated during estrus compared to those treated in diestrus. The interval to estrus was probably extended in the gilts treated during estrus due to luteal function extending beyond treatment. However,

by d 6 post-treatment 88.0% and 96.0% of the gilts treated during estrus and gilts treated during diestrus, respectively, were in estrus.

Serum progesterone levels in control and altrenogest-treated gilts declined ($P < 0.01$) between d 14 and d 17 of the estrous cycle, however, progesterone concentrations were higher in altrenogest-treated gilts on d 14 to 17 than in control gilts. This difference in progesterone levels indicates that either feeding progestin during late diestrus altered or delayed luteolysis or that a metabolite of the progestin was competing with progesterone for antiserum binding sites. Finally, the percentage of gilts farrowing, gestation length, and the number of pigs born was not affected by duration of progestogen feeding.

From these results the authors concluded that 14-d treatment of gilts with altrenogest resulted in a synchronized fertile estrus, regardless of stage of the estrous cycle at the initiation of treatment. Even though 18-d treatment with altrenogest produced more precise synchronization of estrus than treating for 14 d, a 4 d shorter treatment regimen reduced costs and could be more applicable in a commercial operation.

Davis et al. (1985) conducted a study during which estrus was synchronized in gilts with altrenogest fed for 18 consecutive d at 15 mg/d. Following synchronization, gilts were schedule bred (inseminated at a predetermined time after altrenogest feeding) or checked for estrus and then bred. Starting 3.5 d after the last feeding of altrenogest, the gilts in the estrus-checked group were checked twice daily for estrus and inseminated after the second and third detections of estrus. The gilts in the scheduled-AI group were inseminated on d 5, 6, and 7 after the last feeding of altrenogest. Results from this experiment showed that fewer scheduled-AI than estrus-checked gilts exhibited a

synchronized estrus (88 vs. 97%). Furthermore, fewer scheduled-AI than estrus-checked gilts ovulated after altrenogest treatments ($P < 0.05$). The authors suggested that twice daily heat checks by mature boars may have stimulated estrus and ovulation in some of the estrus-checked gilts. However, the proportion of gilts farrowing (73.0% vs. 67.0%), total pigs born (11.0 ± 0.4 vs. 11.3 ± 0.4), and number of pigs born alive (10.1 ± 0.4 vs. 10.5 ± 0.4) were similar ($P > 0.05$) between the estrus-checked and scheduled-AI group, respectively. From these results the authors indicated that scheduled AI breeding programs may be feasible under farm conditions, simplifying breeding herd management.

Martinat-Botte et al. (1990) conducted a similar study evaluating fertility and litter size in gilts synchronized for estrus with altrenogest. Initially, gilts were monitored for estrus to insure cyclicity. Gilts were divided (n = 525) into one of two groups: a control group (n = 281) and a treated group (n = 244) receiving 20 mg of altrenogest daily for 18 d. Results from this experiment showed that 96% of the altrenogest-treated gilts were in estrus between 4 and 7 d after the end of treatment. Following insemination, farrowing rates (88.4% vs. 80.8%, $P < 0.05$) and litter sizes (9.6 vs. 9.1, $P < 0.05$) were significantly increased for the gilts treated with altrenogest compared to gilts that did not receive altrenogest. Similar to the results of the previous studies using progestins to synchronize estrus, this progestin treatment synchronized estrus but also improved fertility and litter size.

Martinat-Botte et al. (1995) conducted a study further evaluating synchronization of estrus in gilts with altrenogest. However, in this study they evaluated the effects on ovulation rate and fetal survival in order to investigate factors that contribute to the increased litter size found in the previous study. Two hundred twenty seven gilts from

the same herd were checked for estrus to insure cyclicity before treatment and assigned to one of two treatment groups: treatment with 20 mg of altrenogest for 18 d (n = 103) or control (n = 124). Gilts were inseminated 12 and 36 h after the onset of their second estrus. Overall, 93% of the gilts in the altrenogest treatment groups showed estrus within 5 to 7 d after treatment. In contrast, 93% of the control gilts showed estrus within 17 to 25 d. Ovulation rate was significantly higher (15.4 ± 0.3 vs. 14.6 ± 0.3; $P < 0.02$) for those gilts treated with altrenogest compared to those receiving control diet. Pregnancy rate at d 22 and d 42 was higher ($P < 0.05$) in the gilts treated with altrenogest (94.2% and 89.3%, respectively) compared to the control group (81.4% vs. 77.4%, respectively). The number of fetuses per pregnant gilt (9.8 ± 0.1 vs. 9.5 ± 0.1; $P > 0.80$) and fetal survival rate were not affected by altrenogest treatment ($P > 0.14$).

The results of several studies verified that altrenogest treatment produced precise synchronization of estrus while improving pregnancy rate. Furthermore, these studies indicate that an increase in ovulation rate in altrenogest-treated gilts lead to an increase in fertility without affecting fetal survivability. Many other studies have been conducted evaluating the ability of altrenogest treatment to synchronize estrus in gilts and sows. Table 3 shows a summary of the results from some of these studies.

P.G. 600

P.G. 600 (Intervet America Inc., Millsboro, DE) is a combination of 400 IU of pregnant mare's serum gonadotropin (PMSG) and 200 IU of human chorionic gonadotropin (hCG). Pregnant mare's serum gonadotropin has biological qualities that mimic the actions of both FSH and LH, whereas hCG mimics the actions of mainly LH.

When injected into prepubertal gilts or weaned sows P.G. 600 is expected to stimulate follicular growth and cause estrus in prepubertal gilts or weaned sows (Britt et al., 1989).

Britt et al. (1989) demonstrated the induction of fertile estrus in prepubertal gilts by treatment with a combination of PMSG and hCG. In their study, 678 gilts from 10 different farms were used from the fall of 1984 to the fall of 1985. On each farm gilts were randomly assigned to one of two groups: gilts receiving an injection of P.G. 600 or gilts receiving no injection. Gilts were monitored for estrus at least once daily for 28 d

Table 3. Use of altrenogest to synchronize estrus in gilts.

Study	n	Dose employed	Duration of treatment	Results
Kraeling et al., 1981	94 gilts and sows	5, 10, 20, 40 mg/d	18 d	100% of the gilts in the 20 and 40 mg/d groups showed estrus within 10 d after treatment cessation
Martinat-Botte et al., 1989	525 gilts	20 mg/d	18 d	96% treated gilts showed estrus; increased farrowing rate and litter size in treated sows
Wood et al., 1992	607 gilts 70 sows	15 mg/d	14 d (gilts) 10 d (sows)	Average d to estrus was decreased in treated gilts and sows
Koutsotheodoros et al., 1998	90 sows (Parity 1)	20 mg	12 d	97% treated sows in estrus vs. 64% untreated within 7 d, increased ovulation rate in treated vs. untreated sows (16.9 vs.15.2, respectively)

after treatment. Gilts detected in estrus were mated naturally once daily throughout the estrus period. Treatment of gilts with P.G. 600 increased (72.9 ± 2.7% vs. 59.5 ± 2.7%) the overall percentage of gilts in estrus within 28 d of injection. The interval from

treatment to onset of estrus was decreased in gilts treated with P.G. 600 compared to non-treated gilts (7.5 ± 0.4 vs. 10.4 ± 0.4% d; $P < 0.05$). Additionally, 57.5% of the gilts treated with P.G. 600 were detected in estrus within the first week after treatment, however, in the control group, only 58.1% of the gilts were in estrus by d 21. Lastly, farrowing rate, litter size, number of pigs weaned, and rebreeding performance after weaning were not different between treatment groups.

Britt et al. (1989) suggested that using P.G. 600 could decrease the interval-to-estrus in prepubertal gilts and increase the precision of synchrony of estrus. Having more gilts exhibit estrus at predicted times allows for more efficient scheduling of breeding and farrowing facilities and allows successful introduction of gilts into weaned sow groups to be bred.

Knox et al. (2000) evaluated the effect of sc vs. i.m. administration of P.G. 600 on estrual and ovulatory responses of prepubertal gilts. Gilts (n = 184) were divided into one of three treatment groups: 1) P.G. 600 s.c. in the flank, 2) P.G. 600 i.m. in the neck 3) no treatment (control). Following treatment, gilts were housed in group pens where they were checked for estrus once daily using fence-line contact with a mature boar. Estrus detection continued for 17 d after the initiation of treatment. On d 17, gilts were slaughtered and ovaries were removed to evaluate the presence of CL, cystic follicles, and cystic CL. A higher ($P < 0.01$) proportion of the gilts receiving sc P.G. 600 (76.0%) expressed estrus compared to those receiving i.m. P.G. 600 (52.0%). However, only 15.0% of control gilts exhibited estrus within 17 d. The interval from initiation of treatment to estrus was reduced ($P < 0.01$) by treatment with P.G. 600 (4.6 d) compared to controls (5.9 d). Both the s.c P.G. 600 administration (86.0%) and i.m. P.G. 600 administration (77.0%) induced more ($P < 0.01$) gilts to ovulate compared to controls

(18.0%). Finally, there were no significant differences between treatment groups for the number of CL, cystic follicles, or number of cystic CL present.

The results of Knox et al. (2000) indicated that a greater number of gilts that received a s.c injection of P.G 600 showed estrus compared to gilts that received an i.m. injection of P.G. 600. However, both the s.c. and i.m. treatment with P.G. 600 were associated with significantly higher percentages of gilts expressing estrus and ovulating and with a decreased time to estrus when compared to control gilts under the same management. Therefore, the authors suggested that treating gilts with P.G. 600 could be a beneficial treatment to induce estrus in females that do not respond to boar exposure, regrouping, or relocation as a means to initiate estrus.

Tilton et al. (1995) evaluated the response of prepubertal gilts to hormonal therapy with P.G. 600 at 140 d of age. Gilts (n = 120) in this study were assigned to one of two groups: treatment with P.G. 600 (5 mL) or no treatment. All gilts were exposed to boars twice daily in order to detect estrus. Blood samples were taken before injection and 48 h after the first detection of estrus to evaluate progesterone levels. Finally, all gilts were laparotomized within 14 d after treatment to determine ovulation rate and number of follicular cysts present. A higher ($P < 0.05$) percentage of gilts treated with P.G. 600 showed estrus within 5 d (69.6%) compared to controls (29.6%). Additionally, a higher percentage of the P.G. 600-treated gilts showing estrus ovulated (99.4%) within 14 d of treatment compared to controls showing estrus (90.4%). However, there was no difference ($P > 0.05$) in ovulation rate between treatment groups. Progesterone levels were similar before treatment for both groups; however, they tended to increase ($P = .13$) in the gilts treated with P.G. 600 (5.9 ng/mL) 48 h after first detection of estrus compared to controls (2.4 ng/mL). Finally, a higher percentage ($P < 0.05$) of gilts treated with P.G

600 (79.0%) had follicular cysts present compared to controls (12.0%). The authors suggested that that P.G. 600 was successful at initiating estrus and ovulation in prepubertal gilts.

Treatment with P.G. 600 has also been used to accelerate the onset of estrus in weaned sows since gonadotropin secretion is suppressed throughout lactation due to suckling (Estienne and Hartsock, 1997). Additionally, P.G. 600 has been used in sows to overcome seasonal infertility resulting from increased ambient temperatures throughout the summer and fall. Typically sows eat less in extremely hot temperatures, therefore they lose weight and body condition, which results in an increase in return to estrus interval after their pigs are weaned (Bates et al., 1991).

Estienne and Hartsock, (1997) conducted a study evaluating the effect of exogenous gonadotropins on the weaning-to-estrus interval in sows. Sows with an average parity of 4.6 nursing 8.6 ± 0.2 pigs were weaned and assigned to one of two treatment groups: 1) 5 mL i.m. injection of P.G. 600, or 2) an injection of 0.9% saline. Sows were monitored for estrus at 24-h intervals in the presence of a mature boar. Sows were inseminated at 0 and 24 h after the first detection of standing estrus. A greater number ($P < 0.05$) of the sows treated with P.G. 600 (97.1%) were in estrus within 7 d after weaning compared to those that received saline (82.9%). Additionally, those sows treated with P.G. 600 had a decreased ($P < 0.01$) treatment-to-estrus interval (3.8 ± 0.1 d vs. 4.5 ± 0.1 d). However, a higher percentage ($P < 0.07$) of the sows treated with saline (96.6%) farrowed compared to those treated with P.G 600 (82.3%). The authors hypothesized that farrowing rate was decreased due to low ovulation rates in some of the P.G. 600 treated sows.

Bates et al. (1991) conducted a study evaluating the performance of sows treated with P.G. 600 at weaning in the summer. In this study sows from eight different herds and three states (n = 592), and ranging in parity from one to three, were assigned to receive P.G. 600 or no treatment at weaning. Sows were exposed to mature boars once daily to detect estrus. Following first detection of estrus, sows were mated each day that they were receptive to a boar. Overall, the percentage of sows achieving estrus within 28 d of weaning was no different between treatment groups. However, there was a decrease ($P < 0.06$) in the number of days to first estrus after treatment in the first (6.0 vs. 7.8 d) and second parity (4.8 vs. 6.4 d) sows compared to controls. Additionally, there was a decrease ($P < 0.06$) in the percentage of anestrus first parity sows in the P. G. 600 treated group (15.6%) compared to controls (29.2%). There was no difference between control sows and P.G. 600-treated sows in parity three group for days to first estrus after treatment (5.5 vs. 5.6 d, respectively) or the percentage of anestrus sows (8.2% vs. 10.4%, respectively). Finally, in contrast to the results of the later study by Estienne and Hartsock (1997), overall the number of pigs born alive was lower ($P = 0.02$) for P.G. 600-treated gilts (10.10) than for control gilts (10.55). From these results the authors suggested that treatment of first and second parity sows with P.G. 600 will induce fertile estrus and reduce weaning-to-estrus intervals during the summer or during other times when estrus initiation is delayed.

Regu-mate/P.G. 600

Previously mentioned studies demonstrated that an orally-active progestin (altrenogest) fed at varying rates from 15 to 20 mg/d for 14 to 18 d was successful at synchronizing estrus in cycling gilts. Additionally, studies previously mentioned indicated that an injection of a combination of 400 IU of PMSG and 200 IU of hCG (i.e.,

P.G. 600) was successful at inducing fertile estrus in prepubertal gilts as well as weaned sows. However, few studies have been conducted to evaluate the effect of pretreatment of randomly cycling or prepubertal gilts with altrenogest followed by an injection of P.G. 600 at the last feeding of altrenogest on synchronization of estrus. Administration of exogenous gonadotropins at the termination of progestin therapy may decrease the interval to estrus after treatment as well as increase ovulation rate in gilts.

Ziecik et al. (1987) evaluated the effect of altrenogest feeding followed by PMSG and hCG injections on the preovulatory LH surge and ovarian hormone secretion in gilts. Gilts were fed altrenogest at a rate of 15 mg/d for 14 d, followed by an injection of 750 IU of PMSG 24 h after the last feeding of altrenogest. Seventy-two h after the PMSG injection gilts were assigned to one of four hCG-injection treatment groups: saline (n = 6), 500 IU hCG (n = 6), 1,000 IU hCG (n = 6), and 1,500 IU hCG (n = 6). All groups treated with hCG showed a decrease ($P < 0.01$) in plasma concentrations of E_2 at 11, 17, and 23 h after hCG injection. Additionally, the hCG-treated animals had elevated P_4 concentrations 60 h after hCG injection (3.1 ± 0.5, 3.4 ± 0.72, and 3.1 ± 0.10 ng/ml in the 500 IU, 1,000 IU and 1,500 IU treated groups respectively vs. 0.9 ± 0.08 ng/mL in the control group). Therefore, ovulatory doses of hCG (500 to 1,500) were able to decrease E_2 immediately after injection and allow P_4 production from newly formed CL to start.

Estienne et al. (2000) evaluated the effects of P.G. 600 on the onset of estrus and ovulation rate in gilts pre-treated with altrenogest. Three different experiments were performed to assess the effect of the altrenogest/P.G. 600 estrus synchronization protocol on the onset of estrus and ovulation rate in gilts. In Exp.1 randomly cycling (n = 64) gilts received altrenogest at a rate of 15 mg/d for 18 d, followed by an i.m. injection of either P.G. 600 (n = 32) or deionized water (control; n = 32) 24 h after altrenogest withdrawal.

There were no differences in the percentage of gilts displaying estrus in 7 or fewer d after injections ($P = 0.64$) and the injection-to-estrus-interval ($P = 0.37$) between the P.G. 600-treated gilts (93.8% and 4.1 ± 0.1 d) and controls (90.6% 4.3 ± 0.1 d). However, average ovulation rate was higher ($P < 0.01$) in the P.G. 600-treated gilts (28.8 ± 1.1) when compared with that of control gilts (17.4 ± 1.1).

In a second experiment reported by Estienne et al. (2001), randomly cycling gilts (n = 58) were treated with altrenogest at a rate of 15 mg/d for 18 d followed by i.m. injection with P.G. 600 (n = 29) or deionized water (n = 29) 24 h after altrenogest withdrawal. Gilts were bred via AI 12 and 24 h after first detection of estrus. Similar to the previous experiment, there were no differences in the percentage of gilts displaying estrus within 7 d after injections ($P = 0.45$) or the average interval from injection-to-estrus ($P = 0.27$) in the gilts treated with P.G. 600 (82.7% and 4.0 ± 0.1 d) or the control group (89.7% and 4.2 ± 0.1 d). Ovulation rate was increased in the gilts treated with P.G. 600 (26.2 ± 1.8) compared to those gilts treated with deionized water (18.1 ± 1.7), however, pregnancy rate ($P = 0.71$) and litter size at d 30 postmating ($P = 0.40$) were similar for the P.G. 600-treated gilts (91.7% and 15.6 ± 1.2) and control gilts (88.5% and 14.1 ± 1.2). Finally, P_4 levels were increased in gilts treated with P.G. 600 on d 7 after treatment (41.2 ± 3.8 vs. 27.1 ± 3.8 ng/mL; $P = 0.02$). However, progesterone concentrations were similar between treatment groups on d 28 after treatment (31.6 ± 2.9 and 31.8 ± 2.9 ng/mL; $P = 0.96$).

In a third experiment performed by Estienne et al. (2001) prepubertal gilts were fed a control diet or a diet providing altrenogest at the rate of 15 mg/d for 18 d. All gilts received an i.m. injection of P.G. 600 24 h after the last feeding of altrenogest. The percentage of gilts displaying estrus within 7 d ($P = 0.49$) and the injection-to-estrus-

interval ($P = 0.69$) were similar between the altrenogest fed gilts (95% and 4.3 ± 0.2 d) and control gilts (88.9% and 4.2 ± 0.2 d). Additionally, ovulation rate was similar ($P = 0.38$) between those gilts fed altrenogest (16.6 ± 1.6) and those gilts that were not fed altrenogest (14.4 ± 1.8).

When combined, the results from the first two experiments reported by Estienne et al. (2001) indicated that altrenogest was successful at synchronizing estrus in randomly cycling gilts. Treatment with P.G. 600 24 h after withdrawal of altrenogest did not affect the number of gilts showing estrus within 7 d of treatment or the injection-to-estrus-interval. Ovulation rate was increased after injection with P.G. 600, but litter size at d 30 was not. Increasing ovulation rate without increasing litter size at d 30 postmating was probably not due to spatial capacity of uteri limiting development of a high number of embryos. It has been suggested that in swine, uterine capacity is not limiting during the first 25 to 30 d of gestation. Perhaps since P.G. 600 increased ovulation rate to > 22, a proportion of the eggs released were primary oocytes (Pope, 1994). Even though primary oocytes are penetrated by spermatozoa they do not undergo activation, therefore, they cannot be fertilized (Polge and Dziuk, 1965; Hunter, 1966). After mating, early in the estrus period, sperm are stored in the caudal 1 to 2 cm of the oviduct isthmus or sperm reservoir for 18 to 30 h or more until a pre-ovulatory phase of activation and release. During this interval of storage, suppression of membrane vesiculation around the acrosome and suppression of hyperactive motility in the flagellum occurs. Following this, a peri-ovulatory stimulation of the acrosome and hyperactivation of the flagellum occurs, which results in the characteristic whiplash motion of the flagellum that allows for penetration of egg membranes. The process of capacitation is interpreted as being completed by a peri-ovulatory endocrine influence of the Graafian follicles on the tubal

milieu. Thus, spermatozoa awaiting fertilization in the oviduct achieve their full fertilizing potential at the time that the secondary oocyte is released by the female into the genital tract. Failure of a proportion of the eggs to be fertilized in superovulated animals, as reported by Zavy and Geisert (1994), may be associated with an initial depletion of the functional sperm reservoirs and a subsequent shortage of viable sperm cells in the proximal section of the oviduct during the protracted period of ovulation that occurs in superovulated animals. The first follicle ovulated is presumed to activate the isthmus reserves of sperm cells. Since capacitated sperm are fragile, unstable, short-lived cells it is possible that eggs released from later maturing follicles in the superovulatory hierarchy may not have been exposed to competent sperm cells (Zavy and Geisert, 1994).

However, we suggest that P.G .600 alters the timing of ovulation in altrenogest fed gilts such that breeding at 12 and 24 h after first detection of standing estrus may not be the most appropriate breeding regimen since it has been suggested that the most optimum time for insemination is 0 to 24 h before ovulation occurs in order to achieve ideal farrowing rates.

In summary, the research presented suggested that the orally active progestin altrenogest was successful at synchronizing estrus. Most gilts displayed estrus within seven d after treatment withdrawal and performance was not negatively affected. Additionally, the research presented suggested that P.G. 600 is successful at initiating estrus and ovulation in prepubertal gilts and decreased the weaning-to-estrus interval in sows, especially during hot periods of the year. Research combining the use of altrenogest and P.G. 600 has shown that these two products are successful at synchronizing estrus and increasing ovulation rate in gilts. Altrenogest was successful at synchronizing estrus in randomly cycling gilts within 7 d and an injection of P.G. 600 24

h after the last feeding of altrenogest was effective in increasing ovulation rate; however, the injection did not affect the injection-to-estrus interval. Even though ovulation rate was increased in gilts that received P.G. 600 in these studies, litter size at d 30 was unchanged. Perhaps this phenomenon is due to P.G. 600 altering the time of ovulation, egg quality, or semen quality at the point of fertilization.

Research has shown that optimal insemination time in gilts and sows in order to achieve ideal fertilization rates is < 24 h before ovulation. Additionally, ovulation has been found to occur at approximately 70% of the duration of estrus. However, researchers have found that predictability of ovulation time in order to inseminate at the optimum time is difficult when using estrus duration or hormone profiles as a guide for prediction.

Recently, transrectal ultrasonography has been used to determine ovulation timing in relation to estrus duration and to investigate follicular dynamics as a means of predicting ovulation timing. With more accurate prediction of ovulation timing, swine producers can inseminate at a more optimum time and thus increase farrowing rates.

In conclusion, having successful estrus synchronization regimens can increase the reproductive efficiency of swine herds. Successful prediction of estrus timing through synchronization allows producers to better utilize their gestation and farrowing facilities and allows them to more efficiently introduce replacement gilts into sow breeding groups after weaning.

CHAPTER II: EFFECT OF P.G. 600 ON THE TIMING OF OVULATION IN GILTS TREATED WITH REGU-MATE

Abstract

We previously reported that ovulation rate, but not pregnancy rate or litter size at d 30 post-mating, was enhanced by gonadotropin treatment (P.G. 600; Intervet America Inc., Millsboro, DE) in gilts fed a progestin (Regu-mate; Intervet America Inc.) to synchronize estrus. We hypothesized that P.G. 600 altered the timing of ovulation, therefore, mating gilts 12 and 24 h after first detection of estrus, as is common in the swine industry, may not have been the optimum breeding regimen. The objective of this study was to determine the effect of P.G. 600 on the timing of ovulation in gilts treated with Regu-mate. Randomly cycling, crossbred gilts (5.5 m of age, 117 kg BW, and 14.7 mm BF) were fed rations with Regu-mate (15 mg/d) for 18 d. Twenty-four h after Regu-mate withdrawal, gilts received i.m. P.G. 600 (n = 25) or saline (n = 25). Gilts were checked for estrus at 8 h intervals. After first detection of estrus, trans-rectal ultrasonography was performed at 8 h intervals to determine the time of ovulation. Gilts were slaughtered 9 to 11 d after the onset of estrus to determine ovulation rate. All gilts displayed estrus by 7 d after treatment with P.G. 600 or saline. Compared with saline, P.G. 600 increased ($P = 0.07$) ovulation rate (14.8 $\pm$ 1.1 vs. 17.5 $\pm$ 1.0, respectively). The interval from injection-to-estrus (110.9 $\pm$ 2.7 h vs. 98.4 $\pm$ 2.7; $P < 0.01$) and injection-to-ovulation (141.9 $\pm$ 3.2 h vs. 128.6 $\pm$ 2.8; $P < 0.01$) were decreased in gilts treated with saline compared with gilts treated with P.G. 600. Estrus duration (54.4 $\pm$ 2.3 vs. 53.7 $\pm$ 2.5 h; $P = 0.83$), the estrus-to-ovulation interval (30.2 $\pm$ 2.0 vs. 31.7 $\pm$ 2.2 h; $P = 0.62$) and the time of ovulation as a percentage of duration of estrus (55.8 $\pm$ 2.7 vs. 57.5 $\pm$

3.0%; $P = 0.67$) were similar for the P.G. 600 and saline-injected gilts, respectively. In summary, P.G. 600 advanced the onset of estrus and ovulation following termination of Regu-mate treatment and increased ovulation rate. However, treatment of gilts with P.G. 600 had no effect on the timing of ovulation relative to the onset of estrus.

Introduction

The ability to synchronize estrus in randomly cycling replacement gilts would improve reproductive efficiency of a swine herd. A combination of the orally active progestin altrenogest (15 mg/d for 18 d) (Regu-mate, Intervet America Inc., Millsboro, DE) and the gonadotropin product P.G. 600 (Intervet America Inc.) given 24 h after the last feeding of progestin has been successful in synchronizing estrus and increasing ovulation rate in cycling gilts (Estienne et al., 2001; Estienne and Harper, 2002). However, despite increased ovulation rates in P.G. 600-treated gilts, pregnancy rate and the number of live embryos at d 30 postmating were not different from gilts treated with Regu-mate alone (Estienne et al., 2001). According to Kemp and Soede (1996), ovulation occurs at approximately 71% of the duration of estrus and breeding sows 0-24 h prior to ovulation resulted in fertilization rates greater than 90 %. Furthermore, a greater number of sows bred < 23 h before ovulation farrowed compared to sows bred > 24 h before ovulation (Knox et al., 2001). Therefore, one possible explanation of our previous findings (Estienne et al., 2001) is that P.G. 600 altered the timing of ovulation and breeding Regu-mate fed gilts 12 and 24 h after first detection of estrus was not the optimum breeding regimen. Therefore, the objective of this study was to determine the effect of P.G. 600 on the timing of ovulation in Regu-mate-fed gilts.

Materials and Methods

General. The experiment was conducted at the Virginia Tech-Tidewater Agricultural Research and Extension Center in Suffolk, VA and took place during the months of June, July, and August 2003. Randomly cycling gilts (Hamline, National Pig Development [Roanoke Rapids, NC] x Landrace x Yorkshire) (n = 50), approximately 5.5 m of age were utilized. At the time of the experiment, gilts weighed an average of 117 ± 6.1 kg and had a mean backfat thickness of 14.7 ± 2.4 mm as determined by ultrasonography (Sono-grader, Renco Inc., Minneapolis, MN) at the tenth rib. Gilts were housed in a passively ventilated building with partially slatted concrete floors. Gilts were penned in groups of eight or nine (2.6 x 5.2 m^2 floor space/pen). Throughout the experiment, gilts were fed a ration that met or exceeded nutrient recommendations as put forth by the NRC (1998) at a rate of 2 kg/hd/d. Gilts had ad libitum access to water via nipple waterers.

Protocol. The protocol was approved by the Institutional Animal Care Committee. Gilts were randomly assigned to one of two treatments: I. Treatment with Regu-mate (15 mg/d) for 18 d followed by treatment with P.G. 600 (400 I.U. PMSG and 200 I.U. hCG) 24 h after the last feeding of Regu-mate (n = 25), and II. Treatment with Regu-mate (15 mg/d) for 18 d followed by an injection of saline 24 h after the last feeding of Regu-mate (n = 25). Equal numbers of gilts in each pen received P.G. 600 or saline injections.

Starting 2 d after administration of either P.G. 600 or saline, gilts were monitored for estrus thrice daily at 0800, 1600, and 2400 in the presence of a mature boar. Gilts displaying estrus were moved into gestation crates (0.6 x 2.1 m^2) in a passively ventilated, curtain-sided barn with partially slatted concrete floors. Gilts continued to be

monitored for estrus thrice daily to determine the duration of estrus. Gilts were scanned using transrectal ultrasound (Aloka 500V, Corometrics Medical Systems, Inc., Wallingford, CT) with a 7.5 mhz linear probe on an angled probe extension (2 mm poly-vinyl coating [PVC] pipe approximately 61 mm long) every 8 h to determine timing of ovulation. Ovulation timing was defined as the midpoint between the last observation of a complete cohort of preovulatory follicles and the first observation of the absence of preovulatory ovarian follicles (Lucy, 1999). Ultrasound images were captured on VHS videotape so that the average size of the ovulatory follicles could be measured. Average follicle size was determined by averaging the size of the 2 largest follicles on the last ultrasound image recorded prior to ovulation.

At nine to 11 d after the onset of estrus, blood was collected via jugular venipuncture. Blood was allowed to clot overnight at 4^{o}C. Following centrifugation, serum was harvested and then stored at –20^{o}C until progesterone concentrations were determined via RIA (Tarraf and Knight, 1995). The intraassay CV averaged 4.5 % and the assay sensitivity was 0.02 ng/mL of serum. Gilts were then slaughtered using a captive bolt pistol followed by exsanguination. Reproductive tracts were collected and ovaries were removed. Ovaries were weighed and corpora lutea (CL) were excised and weighed. Ovulation rate was determined by counting the number of CL. The remaining ovarian tissue was minced and blotted and weight of follicular fluid was determined. Follicular and luteal cysts were classified as previously reported (Kraeling et al., 1981). Follicles with a diameter of 12 mm or greater and fluid filled with little or no luteinization were classified as follicular cysts. Ovarian structures 10 mm or greater and fluid filled with heavy luteinization were classified as luteal cysts. Gilts were classified

as “cystic” if the number of follicular and (or) luteal cysts exceeded the number of CL (Kraeling et al., 1981).

Statistical Analysis Data were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). P.G. 600 or saline injection to estrus interval, estrus duration, estrus to ovulation interval, injection to ovulation interval, the time of ovulation as a percentage of the duration of estrus, and ovarian data were compared using ANOVA with treatment and pen of origin as main effects. Individual means were compared using the PDIFF option of the GLM procedure of SAS.

Results

There was no effect of pen of origin ($P > 0.1$) for any of the variables evaluated in this project. All gilts displayed estrus within 7 d after administration of P.G. 600 or saline. The average injection to estrus interval was decreased ($P = 0.002$) for P.G. 600-treated gilts compared to saline- treated gilts. Mean estrus duration was similar ($P = 0.83$) for P.G 600- treated gilts and saline- treated gilts. The average interval from the onset of estrus to ovulation was similar ($P = 0.62$) for gilts treated with P.G. 600 compared to gilts receiving saline (Table 4).

The mean injection to ovulation interval was shorter ($P < 0.01$) in gilts treated with P.G. 600 than in gilts treated with saline. Time of ovulation as a percentage of the duration of estrus was similar ($P = 0.67$) between treatment groups.

Ovulation rate was increased ($P = 0.07$) for gilts receiving P.G. 600 compared with those receiving saline. There was no difference ($P = 0.71$) in progesterone concentrations measured 9 to 11 d after estrus for gilts treated with P.G. 600 or saline. Mean ovarian weight and mean follicular fluid weight tended to be greater for gilts

Table 4. Mean timing of estrus and ovulation in Regu-mate-pretreated gilts (15 mg/d for 18 d) that received P.G 600 (400 I.U. PMSG and 200 I.U. hCG) or saline (controls) 24 h after withdrawal of Regu-mate

	Treatment Group			
Item	P.G. 600	Saline	**SE**	***P*-value**
No. gilts	25	25	---	---
Gilts in estrus within 7 d after injection (%)	100	100	---	---
Injection to estrus interval (h)	98.4	110.9	2.7	0.002
Estrus duration interval (h)	54.4	53.7	2.5	0.83
Estrus to ovulation interval (h)	30.2	31.7	2.2	0.62
Injection to ovulation (h)	128.6	141.9	3.2	0.003
Time of ovulation as a percentage of the duration of estrus (%)	55.8	57.5	3.0	0.67

treated with P.G. 600 compared to those receiving saline ($P = 0.09$ and $P = 0.10$ respectively). Mean CL weight ($P = 0.29$) and ovulatory follicle size ($P = 0.69$) were similar between gilts treated with P.G. 600 or saline. The number of luteal cysts was similar ($P = 0.57$) for gilts treated with P.G. 600 or saline. More gilts treated with P.G. 600 (n = 8) had follicular cysts present on their ovaries than gilts treated with saline (n = 0) ($P < 0.05$). However, none of the gilts in the experiment were classified as cystic.

Discussion

Previous results from our laboratory indicated that Regu-mate was successful in synchronizing estrus in randomly cycling gilts (Estienne et al., 2001; Estienne and Harper, 2002). In those studies Regu-mate was provided in feed for 14 or 18 d at a rate of 15 mg/d. When the progestin-fed gilts were administered P.G. 600 24 h after withdrawal of Regu-mate ovulation rate was increased compared to gilts treated with

Regu-mate only. However, pregnancy rate and litter size at d 30 was not affected by P.G. 600 treatment.

Table 5. Ovarian characteristics and P_4 concentrations of Regu-mate-pretreated gilts (15 mg/d for 18 d) that were in estrus in ≤ 7 or less d after i.m. administration of P.G. 600 (400 I.U. PMSG and 200 I. U. hCG) or saline (controls).

	Treatment Group			
Item	P.G. 600	Saline	**SE**	***P*-value**
No. gilts	24[a]	22[a]	---	---
Ovarian wt. (g)	9.06	7.96	0.45	0.09
Follicular fluid wt. (g)	2.36	1.81	0.23	0.10
Avg. CL wt. (g)	0.47	0.49	0.02	0.29
No. CL	17.54	14.84	1.05	0.07
No. follicular cysts/gilt	0.44	0.01	0.09	0.004
No. luteal cysts	0.08	0.03	0.06	0.57
Ovulatory follicle size*	7.56	7.47	0.17	0.69
Progesterone ng/mL serum**	28.6	29.7	2.1	0.71

[a] One gilt in the P.G. 600 group and 3 gilts from the saline treated group were withdrawn from the study due to the presence of ileitis.
* Ovulatory follicle size was measured using transrectal real-time ultrasonography (Aloka 500V, Corometrics Medical Systems, Inc., Wallingford, CT).
** Blood samples were taken 9 to 11 d after detected estrus to determine P_4 concentrations

Kemp and Soede (1996), Nissen et al. (1997), and Mburu et al. (1995) reported that ovulation occurs approximately at 70 to 72% of the duration of estrus in mixed parity sows and cyclic gilts. In these same studies, sows that were bred < 24 h before ovulation occurred had significantly higher fertilization rates than sows bred > 24 h prior to ovulation or after ovulation had taken place. Thus, we hypothesized that if P.G. 600 treatment increased ovulation rate, but did not increase litter size, P.G. 600 may be altering the timing of ovulation in Regu-mate fed gilts such that breeding at 12 and 24 h

after first detection of standing estrus was not the optimum breeding regimen. In the current study, the effect of P.G. 600 on the timing of ovulation in gilts treated with Regu-mate was examined.

Randomly cycling gilts treated with Regu-mate displayed a synchronized estrus within 7 d after withdrawal of the progestin. The average interval from Regu-mate withdrawal to estrus was approximately 5.6 d (approximately 4.8 d after saline injection) in our study. These results are similar to those reported by Stevenson and Davis (1982) who indicated that feeding Regu-mate for 14 or 18 d (15 mg/d) synchronized estrus within 5 d after progestin withdrawal in 84.1% of the treated gilts. Davis et al. (1985) found that feeding Regu-mate (15 mg/d for 18 d) was successful at synchronizing 97% of the randomly cycling gilts within 14 d after Regu-mate withdrawal if the gilts were checked for estrus in the presence of a mature boar. However, if the gilts were schedule bred without heat detection, only 88% of the animals showed estrus within 14 d after Regu-mate withdrawal.

It is well documented (Britt et al., 1989, Tilton et al., 1995, Knox et al., 2000) that P.G. 600 is successful at initiating the onset of estrus and ovulation in prepubertal gilts. Additionally, studies (Bates et al., 1991; Estienne and Hartsock, 1997) have shown that treating sows at weaning with P.G. 600 will increase the number of sows returning to estrus within 7 d after weaning and decrease the weaning-to-estrus interval. However, to our knowledge, our previous studies (Estienne et al., 2001; Estienne and Harper, 2002) were the first during which randomly cycling gilts were treated with P.G. 600 after Regu-mate therapy. In those studies a large percentage of randomly cycling gilts that were pretreated with the progestin displayed estrus and ovulated after P.G. 600 treatment. However, the percentages of Regu-mate-fed gilts displaying estrus in $\leq$ 7 d and the

interval from injection-to-estrus were similar after P.G. 600 or deionized water injections (Estienne et al., 2001). In contrast to our previous studies (Estienne et al., 2001), P.G. 600 decreased the time from injection-to-estrus in Regu-mate-fed gilts in the current experiment. Moreover, the timing of ovulation was earlier in Regu-mate fed gilts receiving P.G. 600 compared with gilts receiving Regu-mate alone. In this study, gilts receiving P.G. 600 were in estrus on average about 13 h before gilts that received saline injections and ovulated 13 h before controls. The difference between the current study and our previous research may be related to the difference in frequency of estrus detection. Perhaps in our previous study (Estienne et al., 2001), the time of onset of estrus and interval from injection to estrus were less precise as gilts were monitored for estrus only twice daily and gilts in the current study were monitored for estrus thrice daily. Almeida et al. (2000) found increased variation in the time of ovulation after the onset of estrus in gilts that were monitored for estrus once and twice a day compared to those gilts monitored for estrus 4 times a day (27 - 72 h, 27 - 63 h, and 30 - 60 h, respectively).

Estrus duration was not affected by treatment of Regu-mate-fed gilts with P.G. 600. Previous studies found that typical estrus duration is 52 to 56 h in randomly cycling gilts or weaned sows (Mburu et al., 1995; Kemp and Soede, 1996, Almeida et al., 2000). However, in those studies estrus was detected using the back-pressure test in the presence of a mature boar every 4 or every 6 h to increase the precision of detecting the onset and completion of estrus. In a study similar to ours in which estrus was checked thrice daily in the presence of a mature boar, Nissen et al. (1997) found that duration of estrus in weaned sows was approximately 60 h. However, our results are consistent with the studies that detected estrus 4 to 6 times daily as we found the average duration of estrus

to be 54 to 55 h in Regu-mate fed gilts (Mburu et al., 1995; Kemp and Soede, 1996, Almeida et al., 2000).

The interval from estrus-to-ovulation was not affected by treatment of Regu-mate-fed gilts with P.G. 600. However, the interval from estrus-to-ovulation tended to be shorter in our study compared to previous studies that evaluated timing of ovulation using real-time ultrasonography in randomly cycling gilts or weaned sows (Mburu et al., 1995; Kemp and Soede, 1996, Almeida et al., 2000). We found the average interval from estrus-to-ovulation to be 31 h compared to previous studies that suggested that the estrus-to-ovulation interval was 37 to 44 h (Mburu et al., 1995; Kemp and Soede, 1996, Almeida et al., 2000). Previous studies have shown a wide variation among individual gilts or sows and among groups of gilts or sows on different farms in the interval from estrus-to-ovulation. Variation in this data has been linked to age, housing type, or the frequency of ultrasonography. Additionally, Kemp and Soede (1996) reported that the weaning to estrus interval was negatively related to estrus duration in weaned sows. Perhaps gilts that show estrus on d 4, 5, or 6 after progestin withdrawal may have shorter intervals from estrus to ovulation compared with gilts that show estrus on d 2 or 3 after treatment. Moreover, the lower estrus-to-ovulation interval in our study compared with others (Mburu et al., 1995; Kemp and Soede, 1996, Almeida et al., 2000) could be related to the progestin treatment (Regu-mate) that all gilts in our study received to synchronize estrus.

The time of ovulation as a percentage of the duration of estrus in Regu-mate-fed gilts was not affected by P.G 600 treatment. Similar to the interval from estrus-to-ovulation, the timing of ovulation as a percentage of the duration of estrus in the current study (55 – 58%) was lower than that reported from previous studies (70 – 85%)(Mburu

et al., 1995; Kemp and Soede, 1996, Almeida et al., 2000). Again, the variation among studies could be related to differences in age of the sows or gilts used. Almeida et al. (1999) reported that gilts ovulate later in estrus than sows. Additionally, differences could be related to the frequency of ultrasonography between our study and previous studies (Mburu et al., 1995; Kemp and Soede, 1996, Almeida et al., 2000). Gilts were transrectally ultrasounded every 8 h in our study compared to every 4 to 6 h in previous studies. Finally, as previously mentioned, progestin treatment (Regu-mate) to synchronize estrus in the gilts in our study may be responsible for the decreased interval from the onset of estrus to ovulation. Therefore, the time of ovulation as a percentage of the duration of estrus (interval from onset of estrus to ovulation /estrus duration) would be affected as well.

Similar to our previous work (Estienne et al., 2001), ovulation rate was increased in gilts that received P.G. 600 after withdrawal of Regu-mate compared with gilts that received Regu-mate alone. There was no significant difference in CL weight or number of luteal cysts present between treatment groups. However, there tended to be a trend for increased ovarian weight ($P = 0.09$) and increased follicular fluid weight ($P = 0.10$) in the gilts treated with P.G. 600. Additionally, average ovulatory follicle size was similar between treatment groups. Ovulatory follicle size measured in this study was consistent with that reported by Lucy (1999) and Soede and Kemp (1999) who reported the follicle size to be 7 to 8 mm at the time of ovulation. Furthermore, consistent with our previous study (Estienne et al., 2001) and a study conducted by Tilton et al. (1995), there was an increase in the number of follicular cysts present in gilts treated with P.G. 600. However, the number of follicular cysts did not exceed the total number of CL in any gilt, thus no animals were classified as cystic.

In our previous study (Estienne et al., 2001) in which randomly cycling gilts were treated with Regu-mate, progesterone concentrations 9 to 11 d after estrus were significantly increased in those gilts that received an injection of P.G. 600 compared to those that received saline 24 h after withdrawal of Regu-mate. In that study when gilts were slaughtered to determine ovulation rate, gilts that received P.G. 600 treatment had approximately 12 more CL present compared to the saline-treated gilts. Therefore, it is not surprising that P.G. 600 treatment significantly increased serum progesterone levels. In the current study, gilts treated with P.G .600 had an average of only 3 more CL than saline-treated gilts. This relatively small difference in ovulation rate did not result in a difference in mean serum progesterone levels of gilts treated with P.G. 600 or saline.

Even though the injection-to-estrus interval and injection-to-ovulation interval were decreased in gilts treated with P.G. 600 24 h after the last feeding of Regu-mate, the estrus-to-ovulation interval and the time of ovulation as a percentage of the duration of estrus were not affected by treatment with P.G. 600. Therefore, the findings in our previous study that ovulation rate, but not pregnancy rate or litter size was increased by P.G. 600, in Regu-mate-fed gilts, is probably not attributable to inappropriate timing of mating relative to the onset of estrus.

Our previous finding that P.G. 600 treatment increased ovulation rate without simultaneously increasing litter size at d 30 post mating (Estienne et al., 2001) is unlikely to have been due to limited spatial capacity of the uterus. Uterine capacity is not limiting until 25 to 30 d after gestation (Pope, 1994). Alternatively, it is possible that the large number of the eggs ovulated in the gilts treated with P.G. 600 after Regu-mate withdrawal resulted in a higher rate of unfertilized ova. It has been reported when ovulation rates are > 22 a higher proportion of are primary oocytes undergo ovulation

(Pope, 1994). Since primary oocytes do not undergo activation, they cannot be fertilized (Polge and Dziuk, 1965; Hunter, 1966). Additionally, superovulated animals tend to have a protracted period of ovulation of at least 40 h so there may be a deleterious influence of progesterone secretion from the newly-formed CL on subsequent embryo transport in the oviducts. Furthermore, the first follicle ovulated is presumed to activate the reservoirs of sperm in the isthmus. Capacitated sperm are fragile, short-lived cells, therefore; it is possible that eggs released from later maturing follicles in the superovulatory hierarchy are not exposed to competent sperm (Zavy and Geisert, 1994). While this theory is worthy of consideration, it should be noted that in our current study the period of ovulation was not impacted by P.G. 600 treatment.

In summary, P.G. 600 advanced the onset of estrus and ovulation and increased ovulation rate in Regu-mate treated gilts. However, P.G. 600 had no effect on the timing of ovulation relative to the onset of estrus.

Implications

Having replacement gilts exhibit estrus at a predicted time enhances reproductive efficiency of a swine herd. We previously reported that ovulation rate, but not pregnancy rate or litter size at d 30 post-mating, was enhanced by P.G. 600 treatment in gilts pre-treated with Regu-mate compared with gilts receiving Regu-mate alone. In this experiment we showed that Regu-mate treatment was successful in synchronizing estrus in gilts within 7 d after progestin withdrawal. The progestin, previously marked for horses under the trade name Regu-mate, altrenogest was approved by the FDA and is now available to swine producers under the trade name “Matrix”. Treatment with P.G. 600 treatment resulted in decreased interval from injection-to-estrus and the interval from injection-to-ovulation, while increasing the ovulation rate. However, P.G. 600 did not

alter the timing of ovulation, so the typical regimen of breeding gilts 12 and 24 h after the onset of estrus should not be changed in order to achieve more ideal fertilization rates.

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SUMMARY AND CONCLUSIONS

The goal of this project was to determine the effect of P.G. 600 on timing of ovulation in gilts treated with an orally-active progestin, altrenogest (Regu-mate). Specifically, the authors were trying to explain why gilts receiving Regu-mate (15 mg/d for 18 d) and an injection of P.G. 600 24 h after progestin withdrawal did not have increased litter size at d 30 post-mating even though gilts had increased ovulation rates when compared to control gilts that received Regu-mate only. The authors hypothesized that P.G. 600 altered the time of ovulation so that breeding gilts 12 and 24 h after the onset of standing estrus may not have been the optimum breeding regimen to use.

Results from the experiment showed that there was no difference in the timing of ovulation relative to the onset of estrus or in relation to the duration of estrus for Regu-mate-fed gilts treated with P.G. 600 or saline as determined by transrectal, real-time ultrasonography. However, in this study the authors found that Regu-mate was successful at synchronizing estrus in 100% randomly cycling gilts within 7 d after withdrawal of progestin treatment. Additionally, similar to results from their previous work, the authors found that treatment with P.G. 600 did result in an increase in ovulation rate.

From these results the authors conclude that P.G. 600 does not alter the timing of ovulation in relation to the duration of estrus in gilts. Therefore, the common regimen of breeding gilts 12 and 24 h after the onset of estrus is recommended. However, further research should be conducted to evaluate egg quality in Regu-mate-fed gilts that are "superovulated" with P.G. 600.

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VITA

Brandon Ryan Horsley, son of Don and Diane Horsley, was born on March 16, 1980 in Virginia Beach, Virginia.

He has lived in Virginia Beach all of his life on a farrow-to-finish swine operation and grain farm. He graduated from Kellam High School in June of 1998. He obtained a Bachelor of Science degree in Animal and Poultry Sciences and a minor in Agricultural and Applied Economics from Virginia Polytechnic Institute and State University in May of 2002. That same year he returned to Virginia Polytechnic Institute and State University to pursue the Master of Science degree in Animal and Poultry Sciences with an emphasis on swine reproductive physiology.

He is a current member of the American Society of Animal Science and the National Biological Honor Society, Phi Sigma. Also, he was a member of the 2000 Virginia Tech Intercollegiate Livestock Judging Team and a member of the Block and Bridle Club.

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