Effect of P.G. 600 on the timing of ovulation in gilts treated with altrenogest


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ABSTRACT: We previously reported that ovulation rate, but not pregnancy rate or litter size at d 30 after mating, was enhanced by treatment with P.G. 600 (400 IU of PMSG and 200 IU of hCG, Intervet America, Inc., Millsboro, DE) in gilts fed the orally active progestin, altrenogest (Matrix, Intervet America, Inc.) to synchronize estrus. We hypothesized that in addition to increasing ovulation rate, P.G. 600 may have 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 optimal breeding regimen, and as a consequence, pregnancy rate and litter size were not altered. The objective of the present study was to determine the effect of P.G. 600 on the timing of ovulation in gilts treated with altrenogest. Randomly cycling, crossbred gilts (5.5 mo old, 117 kg BW, and 14.7 mm of backfat) were fed a diet containing altrenogest (15 mg/d) for 18 d. Twenty-four hours after altrenogest withdrawal, gilts received i.m. injections of P.G. 600 (n = 25) or saline (n = 25). Gilts were checked for estrus at 8-h intervals. After first detection of estrus, transrectal 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 vs. 17.5, respectively; SE = 1.1). The intervals from injection to estrus (110.9 vs. 98.4; SE = 2.7 h; P < 0.01) and injection to ovulation (141.9 vs. 128.6; SE = 3.2 h; P < 0.01) were greater in gilts treated with saline than in gilts treated with P.G. 600. Duration of estrus (54.4 vs. 53.7; SE = 2.5 h), the estrus-to-ovulation interval (30.2 vs. 31.7; SE = 2.2 h), and the time of ovulation as a percentage of estrus duration (55.8 vs. 57.5; SE = 3.0%) did not differ 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 altrenogest 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.

Key Words: Gilt, Gonadotropin, Ovulation, Progestin

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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 (Matrix; Intervet America In., Millsboro, DE; 15 mg/d for 18 d) and the gonadotropin product P.G. 600 (400 IU of PMSG and 200 IU of hCG, Intervet America Inc.) given 24 h after the last feeding of altrenogest successfully synchronized estrus in cycling gilts (Estienne et al., 2001; Estienne and Harper, 2002). Despite increased ovulation rates in P.G. 600-treated, altrenogest-fed gilts, however, pregnancy rate and the number of live embryos at d 30 after mating did not differ from that of gilts treated with altrenogest 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 to 24 h before ovulation resulted in fertilization rates greater than 90%. Furthermore, a greater number of sows bred < 23 h before ovulation farrowed compared with sows bred > 24 h before ovulation (Knox et al., 2001). Therefore, one possible explanation of our previous findings that P.G. 600 increased ovulation rate but not litter size at d 30 after mating (Estienne et al., 2001) is that P.G. 600 altered the timing of ovulation in altrenogest-
fed gilts, and mating 12 and 24 h after first detection of estrus was not the optimal breeding regimen. Therefore, the objective of this study was to determine the effect of P.G. 600 on the timing of ovulation in altrenogest-fed gilts.

Materials and Methods

General

The experiment was conducted at the Virginia Tech-Tidewater Agricultural Research and Extension Center in Suffolk, VA, during the months of June, July, and August 2003. Randomly cycling gilts (Hamline National Pig Development; Roanoke Rapids, NC × Landrace × Yorkshire; n = 50), approximately 5.5 mo of age, were used. Gilts weighed an average of 117 ± 6.1 kg and had a mean 10th-rib backfat thickness of 14.7 ± 2.4 mm as determined by ultrasonography (Sono-grader, Renco Inc., Minneapolis, MN). Gilts were housed in a passively ventilated building with partially slatted concrete floors and were penned in groups of eight or nine (2.6 m × 5.2 m floor space per pen). Throughout the experiment, each gilt was fed at a rate of 2 kg/d (as-fed basis), a fortified, corn and soybean meal-based diet that met or exceeded NRC (1998) nutrient recommendations. The diet containing altrenogest was prepared by gradual addition of the appropriate volume of altrenogest product to the gilt diet as it was mixed in a horizontal feed mixer. Final concentration was 7.5 mg/kg to provide 15 mg/d of altrenogest for each gilt. Gilts had ad libitum access to water via nipple waterers.

Protocol

The protocol was approved by the Institutional Animal Care Committee of Virginia Polytechnic Institute and State University. Gilts were assigned randomly to one of two treatments: 1) treatment with altrenogest (15 mg/d) for 18 d followed by i.m. treatment with P.G. 600 24 h after the last feeding of altrenogest (n = 25); and 2) treatment with altrenogest (15 mg/d) for 18 d followed by an i.m. injection of saline 24 h after the last feeding of altrenogest (n = 25). In each pen, four or five gilts received injections of P.G. 600, and four or five gilts received 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 m × 2.1 m) in a passively ventilated, curtain-sided barn with partially slatted concrete floors. Gilts continued to be monitored for estrus three times daily to determine the duration of estrus. Gilts also were scanned using trans-rectal ultrasound (Aloka 500, Corometrics Medical Systems, Inc., Wallingford, CT) with a 7.5-MHz linear probe on an angled probe extension (2-mm polyvinyl-coated [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 videotape, so that the average size of the ovulatory follicles could be measured during playback. Average follicle size was determined by averaging the size of the two largest follicles on the last ultrasound image recorded before ovulation.

On d 9 to 11 after the onset of estrus, blood was collected via jugular venipuncture. Blood was allowed to clot overnight at 4°C. Following centrifugation at 400 × g, serum was harvested and then stored at −20°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. At 9 to 11 d after the onset of estrus, gilts were killed by stunning with 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). Fluid-filled follicles with a diameter of 12 mm or greater and with little or no luteinization were classified as follicular cysts. Fluid-filled ovarian structures with a diameter of 10 mm or greater and with heavy luteinization were classified as luteal cysts.

Statistical Analyses

Data were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The interval from P.G. 600 or saline to estrus, duration of estrus, 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. Percentages of gilts first exhibiting estrus at various times after treatment with P.G. 600 or saline were analyzed using $\chi^2$ analysis.

Results

There was no effect of pen of origin ($P > 0.10$) on any of the reproductive characteristics. The timing of estrus and ovulation in altrenogest-fed gilts treated with P.G. 600 or saline is summarized in Table 1, and the percentages of gilts first exhibiting estrus at various times after treatment are depicted in Figure 1. All gilts displayed estrus within 7 d after administration of P.G. 600 or saline. The average injection-to-estrus interval was decreased ($P < 0.01$) for P.G. 600-treated gilts compared with saline-treated gilts. Mean duration of estrus did not differ for P.G. 600-treated gilts and saline-treated controls. The average interval from the onset of estrus...
Table 1. Mean timing of estrus and ovulation in gilts that received P.G. 600 (400 IU of PMSG and 200 IU of hCG) or saline (controls) 24 h after cessation of altrenogest treatment (15 mg/d for 18 d)a

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment group</th>
<th>P.G. 600</th>
<th>Saline</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of gilts</td>
<td></td>
<td>25</td>
<td>25</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gilts in estrus within 7 d after injection, %</td>
<td></td>
<td>100</td>
<td>100</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Injection-to-estrus interval, h</td>
<td></td>
<td>98.4</td>
<td>110.9</td>
<td>2.7</td>
<td>0.01</td>
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<tr>
<td>Duration of estrus, h</td>
<td></td>
<td>54.4</td>
<td>53.7</td>
<td>2.5</td>
<td>0.83</td>
</tr>
<tr>
<td>Estrus-to-ovulation interval, h</td>
<td></td>
<td>30.2</td>
<td>31.7</td>
<td>2.2</td>
<td>0.62</td>
</tr>
<tr>
<td>Injection-to-ovulation, h</td>
<td></td>
<td>128.6</td>
<td>141.9</td>
<td>3.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Time of ovulation as a percentage of the duration of estrus, %</td>
<td></td>
<td>55.8</td>
<td>57.5</td>
<td>3.0</td>
<td>0.67</td>
</tr>
</tbody>
</table>

aValues are least squares means.

to ovulation did not differ for gilts treated with P.G. 600 compared with gilts receiving saline. 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 between treatment groups.

Ovarian characteristics and serum progesterone concentrations in altrenogest-fed gilts treated with P.G. 600 or saline are summarized in Table 2. Before slaughter, one gilt in the P.G. 600-treated group and three gilts in the control group developed clinical signs of ileitis. These animals were removed from the remainder of the study. Ovulation rate was increased ($P = 0.07$) for gilts receiving P.G. 600 compared with those receiving saline. There was no difference in progesterone concentrations measured in serum collected 9 to 11 d after estrus in gilts treated with P.G. 600 or saline. Mean ovarian weight and mean follicular fluid weight tended to be greater for gilts treated with P.G. 600 compared with those receiving saline ($P = 0.09$ and 0.10, respectively). Mean CL weight and ovulatory follicle size were similar between gilts treated with P.G. 600 or saline. The number of luteal cysts was similar 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 given saline (n = 0; $P < 0.01$), and the number of follicular cysts was greater ($P < 0.01$) for the gonadotropin-treated animals.

Discussion

The efficacy of altrenogest to synchronize estrus in randomly cycling gilts is well documented (for reviews, see Webel and Day, 1982; Day, 1984; Gordon, 1997). Previous results from our laboratory indicated that altrenogest was successful in synchronizing estrus when provided in feed for 14 or 18 d at a rate of 15 mg/d (Estienne et al., 2001; Estienne and Harper, 2002). When P.G. 600 was administered 24 h after withdrawal of altrenogest, ovulation rate was increased compared with gilts treated with altrenogest only (Estienne et al., 2001); however, pregnancy rate and litter size at d 30 were not affected by P.G. 600 treatment.

Kemp and Soede (1996), Nissen et al. (1997), and Mburu et al. (1995) reported that ovulation occurs at 70 to 72% of the duration of estrus in mixed-parity sows. In these same studies, sows that were bred < 24 h before ovulation had significantly higher fertilization rates than sows bred > 24 h before ovulation or after ovulation had taken place. Thus, we hypothesized that if P.G. 600 treatment increased ovulation rate in altrenogest-fed gilts, but it did not increase litter size, the gonadotropin product may have altered the timing of ovulation, such that breeding at 12 and 24 h after first detection of standing estrus was not the optimal breeding regimen. Thus, in the current study, the effect of P.G. 600 on the timing of ovulation in gilts treated with altrenogest was examined.

Randomly cycling gilts treated with altrenogest alone displayed a synchronized estrus within 7 d after with-
The average interval from altrenogest withdrawal to estrus was approximately 5.6 d (approximately 4.8 d after saline injection). These results are similar to those reported by Stevenson and Davis (1982), who reported that 84.1% of altrenogest-treated gilts displayed estrus within 5 d after progestin withdrawal. In that study, altrenogest was fed for 14 or 18 d at a rate of 15 mg/d.

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 pubertal gilts. Additional studies (Bates et al., 1991; Estienne and Hartsock, 1998) 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 altrenogest 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. Nonetheless, the percentages of altrenogest-fed gilts displaying estrus in ≤7 d and the interval from injection-to-estrus did not differ 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 altrenogest-fed gilts in the current experiment. Moreover, the injection-to-ovulation interval was decreased in altrenogest-fed gilts receiving P.G. 600 compared with gilts receiving altrenogest alone. In the present study, gilts receiving P.G. 600 were in estrus on average approximately 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), estimates of the time of onset of estrus and interval from injection to estrus were less precise because gilts were monitored for estrus only twice daily. Gilts in the current study were monitored for estrus three times daily. Almeida et al. (2000) found increased variation in estimates of the time of ovulation after the onset of estrus in gilts that were monitored for estrus once and twice a day compared with those gilts monitored for estrus four to six times a day (27 to 72, 27 to 63, and 30 to 60 h, respectively). Finally, it cannot be discounted that differences in the ability of P.G. 600 to expedite the onset of estrus in altrenogest-fed gilts in our previous study (Estienne et al., 2001) and the current experiment could be due to the different seasons in which the investigations were conducted, the genetics of the experimental animals employed, and/or batches of P.G. 600 used.

Duration of estrus was not affected by treatment of altrenogest-fed gilts with P.G. 600. Previous studies conducted in gilts and sows (Mburu et al., 1995; Kemp and Soede, 1996, Almeida et al., 2000) demonstrated large variation in the duration of estrus and was 52.6 h (range of 30 to 72 h) in randomly cycling gilts (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 6 h to increase the precision of detecting the onset and completion of estrus. Our results are consistent with the studies that detected estrus four to six times daily, as we found the average duration of estrus to be 54 to 55 h in altrenogest-fed gilts (Mburu et al., 1995; Kemp and Soede, 1996; Almeida et al., 2000). In a study similar to ours in which estrus was checked three times daily in the presence of a mature boar, Nissen et al. (1997) reported that duration of estrus in weaned sows was approximately 60 h.

The interval from estrus-to-ovulation was not affected by treatment of altrenogest-fed gilts with P.G. 600; however, the estrus-to-ovulation interval tended to be shorter in our study compared with previous studies that evaluated timing of ovulation using real-time ul-

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Table 2. Ovarian characteristics and progesterone concentrations of altrenogest-pretreated gilts (15 mg/d for 18 d) that were in estrus in ≤7 d after i.m. administration of P.G. 600 (400 IU of PMSG and 200 IU of hCG) or saline (controls)\(^a\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment group</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of gilts</td>
<td>24</td>
<td>22</td>
<td>—</td>
</tr>
<tr>
<td>Ovarian weight, g</td>
<td>9.06</td>
<td>7.96</td>
<td>0.45</td>
</tr>
<tr>
<td>Follicular fluid weight, g</td>
<td>2.36</td>
<td>1.81</td>
<td>0.23</td>
</tr>
<tr>
<td>No. of corpora lutea</td>
<td>17.54</td>
<td>14.84</td>
<td>1.1</td>
</tr>
<tr>
<td>Mean corpora lutea weight, g</td>
<td>0.47</td>
<td>0.49</td>
<td>0.02</td>
</tr>
<tr>
<td>No. of follicular cysts</td>
<td>0.44</td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>No. of luteal cysts</td>
<td>0.08</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Ovulatory follicle size, mm(^b)</td>
<td>7.56</td>
<td>7.47</td>
<td>0.17</td>
</tr>
<tr>
<td>Progesterone, ng/mL serum(^c)</td>
<td>28.6</td>
<td>29.7</td>
<td>2.1</td>
</tr>
</tbody>
</table>

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\(^a\)Values are least squares means.  
\(^b\)Ovulatory follicle size was measured using transrectal real-time ultrasonography (Aloka 500V, Comer Medical Systems, Inc., Wallingford, CT).  
\(^c\)Blood samples were taken 9 to 11 d after gilts were detected estrus to determine progesterone concentrations.
In the current study, however, the incidence of follicular cysts present in gilts treated with P.G. 600 was comparable to that reported by Tilton et al. (1995), Kemp and Soede (1996); however, there was an increase in the number of follicular cysts present in gilts treated with P.G. 600 compared with gilts treated with saline. Additionally, Kemp and Soede (1996) reported that the weaning-to-estrus interval was negatively related to duration of estrus in weaned gilts. 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 (altrenogest) that all gilts in our study received to synchronize estrus.

The time of ovulation as a percentage of the duration of estrus in altrenogest-fed gilts was not affected by P.G. 600 treatment. Similar to the estrus-to-ovulation interval, the timing of ovulation as a percentage of the duration of estrus in the current study (55 to 58%) was lower than that reported from previous studies in sows (70%; Mburu et al., 1995; Kemp and Soede, 1996) and gilts (85%; Almeida et al., 2000). Again, the variation among studies could be related to differences in the age of the sows or gilts used. Additionally, differences could be related to the frequency of ultrasonography. Additionally, Kemp and Soede (1996) reported the weaning-to-estrus interval was negatively related to duration of estrus in weaned sows. 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 (altrenogest) that all gilts in our study received to synchronize estrus.

Similar to our previous work (Estienne et al., 2001), ovulation rate was increased in gilts that received P.G. 600 after withdrawal of altrenogest compared with gilts that received saline. This relatively small difference in ovulation rate did not result in a difference in mean serum progesterone concentrations among groups of gilts or sows on different farms in the interval from estrus-to-ovulation. Variation in these 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 duration of estrus in weaned sows. 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 (altrenogest) that all gilts in our study received to synchronize estrus.

In our previous study (Estienne et al., 2001), in which randomly cycling gilts were treated with altrenogest, progesterone concentrations 9 to 11 d after estrus were increased significantly in those gilts that received an injection of P.G. 600 24 h after withdrawal of altrenogest compared with those that received saline. When gilts were killed to determine ovulation rate, gilts that received P.G. 600 treatment had approximately 12 more CL present compared with the saline-treated gilts. Therefore, it is not surprising that P.G. 600 treatment significantly increased serum progesterone concentrations.

In the current study, gilts treated with P.G. 600 had an average of only three more CL than saline-treated gilts. This relatively small difference in ovulation rate did not result in a difference in mean serum progesterone levels for gilts treated with P.G. 600 or saline.

Although the injection-to-estrus and injection-to-ovulation intervals were decreased in gilts treated with P.G. 600 24 h after the last feeding of altrenogest, 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 altrenogest-fed gilts, were 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 after mating (Estienne et al., 2001) is unlikely to have been due to limited spatial capacity of the uterus. Uterine capacity is not limiting until 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 altrenogest withdrawal resulted in a higher rate of unfertilized ova. It has been reported that when ovulation rates exceed 22 ova, a higher proportion of primary oocytes are ovulated (Hunter, 1966). Because primary oocytes do not undergo activation, they cannot be fertilized (Polge and Dziuk, 1965; Hunter, 1966). Additionally, superovulated animals tend to have a prolonged 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 cells (Zavy and Geisert, 1994). Although this theory is worthy of consideration, it should be noted that in our current study, the period of ovulation was probably not affected by P.G. 600 treatment.
In summary, P.G. 600 advanced the onset of estrus and ovulation and increased ovulation rate in altrenogest-treated gilts; however, P.G. 600 had no effect on the timing of ovulation relative to the onset of estrus.

**Literature Cited**


