Administration of Testosterone from Day 13 of the Estrous Cycle to Estrus Increased the Number of Corpora Lutea and Conceptus Survival in Gilts

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ABSTRACT: The effects of exogenous androgens on the number of corpora lutea (CL) and conceptus survival were examined in crossbred gilts. In Exp. 1, gilts received 1 mg of testosterone per day from d 13 (d 0 = first day of estrus, n = 21) or d 16 until estrus (n = 23). Gilts in the vehicle group received corn oil (n = 20). Gilts were mated and on d 11.5 their concepti and CL were evaluated. In Exp. 2, conceptus survival was examined at the 4- to 8-cell, early blastocyst or hatching blastocyst stages for gilts given vehicle or 1 mg testosterone from d 13 (24 gilts per group). In Exp. 3, gilts received 1 mg of androstenedione (n = 20) or vehicle (n = 18) per day from d 13 to estrus and then were mated and evaluated on d 11.5. Results from Exp. 1 indicated that the number of CL was greater (P < .04) in gilts treated with testosterone from d 13 to estrus than in gilts receiving vehicle (16.4 vs 14.8, respectively). Similarly, the number (P < .01) and recovery rate (P < .04) of blastocysts were greater in gilts treated with testosterone from d 13 to estrus than in gilts treated with testosterone from d 16 to estrus or in gilts receiving vehicle (number, 15.3 vs 12.8 or 12.8; recovery rate, 95 vs 87 or 86%, respectively). Gilts treated with testosterone or vehicle did not exhibit differences (P > .05) in number of normal concepti at the 4- to 8-cell and hatching stages. However, prior treatment with testosterone delayed conceptus death; gilts treated with testosterone had more (P < .01) normal concepti at the intermediate stage (early blastocyst) than those treated with vehicle (treatment × embryo stage interaction, P < .05). In Exp. 3, androstenedione treatment did not influence (P > .10) the number of CL or the number and recovery rates of d-11.5 blastocysts. Treating gilts with testosterone from d 13 of the estrous cycle to the following estrus increased the number of CL and blastocyst survival, perhaps by improving some, as yet unknown, aspect(s) of oocyte quality.

Key Words: Pigs, Testosterone, Ovulation Rate, Blastocyst


Introduction

Administration of testosterone to gilts on d 17 and 18 of the estrous cycle increased ovulation rate and the number of d-11 concepti (Cárdenas and Pope, 1994) and could, theoretically, increase litter size. Exogenous testosterone also increased plasma concentrations of estradiol in gilts (Cárdenas and Pope, 1994) and cows (Kotwica and Williams, 1982). These observations suggested that the effects of testosterone on increasing ovulation rate in gilts might be mediated through the actions of estradiol, because the health and development of follicles are usually associated with amounts of estradiol within the antral fluid (Peters and McNatty, 1980; Hsueh et al., 1984). If the effects of testosterone are mediated by estradiol, then perhaps androstenedione, the other substrate utilized by pig follicles for the synthesis of estradiol (Anderson et al., 1979; Schomberg, 1979; Evans et al., 1981), might produce similar effects to those observed with testosterone.

Stimulation of follicular development with exogenous androgens might, in turn, augment oocyte maturation and improve the proportion of concepti surviving during early gestation. Various investigators have hypothesized that the degree of follicular maturation and oocyte development is related to conceptus survival (Pope et al., 1988; Hunter and Wiesak, 1990). For example, Xie et al. (1990) observed that the diversity of follicular development, in turn, influenced the diversity among littermate concepti.
Some early relationships between the dose of testosterone and number of corpora lutea (CL) or conceptus survival have been described for swine (Cárdenas and Pope, 1994). In the present experiments the effects of different durations of testosterone treatment and the effects of androstenedione on number of CL, conceptus survival, and diversity among littermate concepti were examined in multiestrous gilts.

Materials and Methods

Crossbred gilts (1/2 Duroc × 1/4 Landrace × 1/4 Yorkshire, 6 to 8 mo of age, 110 to 130 kg of body weight initially) were observed at approximately 12-h intervals for estrous behavior in the presence of intact boars. Gilts that exhibited at least one period of estrus were used in the following experiments.

Experiment 1. Gilts were allocated to receive daily intramuscular injections of one of the following treatments: 1) vehicle (corn oil) from d 13 of the estrous cycle (d 0 = first day of estrus) until the first day of the next estrus (n = 10), 2) 1 mg of testosterone (Sigma Chemical, St. Louis, MO) dissolved in corn oil from d 13 to estrus, as above (n = 21), or 3) vehicle on d 13, 14, and 15 and then 1 mg of testosterone from d 16 of the estrous cycle to estrus (n = 23). Gilts that displayed estrus 12 h after the last injection of vehicle or testosterone did not receive additional injections.

Gilts within each treatment group were assigned to receive testosterone dissolved in 1 or 7 mL of corn oil. The 7-mL volume of vehicle was used in previous experiments to facilitate dissolving of larger amounts of testosterone (Cárdenas and Pope, 1994). Characteristics evaluated were not altered by volume of vehicle; therefore, data were pooled for the final statistical analyses. Furthermore, in a small trial performed simultaneously using ovariohysterectomized gilts, plasma concentrations of testosterone at 0, 1, 2, 4, 6, 8, 12, 16, and 24 h after administration of 1 mg of testosterone were not altered (P = .30) by volume (1 or 7 mL, n = 5 gilts/group) of vehicle or the interaction of volume of vehicle with time (P = .77).

In order to decrease variability due to duration of the estrous cycle, 7 out of 64 gilts (3 and 4 gilts that received testosterone beginning on d 13 or d 16, respectively) were excluded from the experiment because their estrous cycles did not fall within the range of 18.0 to 22.0 d. For gilts that remained in the experiment, duration of the estrous cycle of gilts did not differ (P = .32). Estrous cycle durations in vehicle gilts and gilts treated with testosterone beginning on d 13 or d 16 were 19.4 ± .2, 19.3 ± .2, and 19.0 ± .2 d, respectively.

Gilts were mated to boars at 12, 24, and 36 h after onset of estrus and were subsequently ovariohysterectomized on d 11.5. Corpora lutea were counted, dissected from the ovaries, and weighed. Blastocysts were recovered by flushing the uterus with physiological saline (.9% NaCl) within 10 to 15 min after surgery. Before flushing, the mesometrium was separated from the uterus and the tip of each uterine horn was cut approximately .5 cm from the uterine-tubal junction. The uterus was flushed twice by infusing 50 mL (total of 100 mL) of saline into the tip of one horn and recovering the flushing through the tip of the opposite uterine horn. Concepti were examined using a stereomicroscope and then were measured to the nearest millimeter at their largest diameter. Number of filamentous blastocysts entangled during flushing were estimated based on the number of trophoblastic ends. Morphological diversity among littermate blastocysts was estimated by calculating the standard deviation of blastocyst diameter.

Experiment 2. This experiment examined conceptus survival at earlier stages of development than those in Exp. 1. Gilts (n = 24 per group) were administered 1 mg of testosterone or vehicle from d 13 of the estrous cycle until estrus and then mated as described in Exp. 1. This treatment was selected because it increased the number of CL and recovery rates of blastocysts in Exp. 1. Corpora lutea and concepti were evaluated after ovariohysterectomies on d 4.0, 5.5, or 7.0. Concepti were recovered by flushing each ovary and uterine horn three times with 25 mL of modified Tyrode’s medium buffered with Hepes (Hagen et al., 1991). This medium was used instead of saline because it took longer to search and recover the concepti at these earlier stages of development than at the elongating stage (d 11.5).

Morphological development of concepti was determined by microscopic examination at magnifications up to 100×, and then concepti were classified as normal or degenerating. Concepti, which were developing normally, were at the 4- to 8-cell, early blastocyst and hatching blastocyst stages on d 4.0, 5.5, and 7.0, respectively. The early blastocyst stage included the period of blastocoel formation, whereas the hatching stage comprised expanding and hatched blastocysts.

Experiment 3. Gilts were administered 1 mg of androstenedione (Sigma Chemical, n = 20) or vehicle (n = 18) per day from d 13 of the estrous cycle to the first day of their next estrus. Gilts were mated and concepti were recovered on d 11.5 as described in Exp. 1.

Statistical Analyses. Data from Exp. 1 and Exp. 3 were analyzed by one-way ANOVA. Seven gilts, with some or all filamentous blastocysts, that were unmeasurable were not included in the analysis of conceptus diameter in Exp. 1. Recovery rate of concepti was calculated by dividing the number of concepti by the number of CL and multiplying by 100. Because of the possibility that gilts from which no concepti were recovered had total fertilization failure, they (one gilt per group in Exp. 1 and 3) were not
Table 1. Number and weight of corpora lutea (CL) in vehicle- and testosterone-treated gilts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean no. of CL</th>
<th>Mean CL weight (mg) per gilt (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. 1 (n)</td>
<td>Exp. 2 (n)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>14.8 ± 0.5c</td>
<td>13.3 ± 0.4c (20)</td>
</tr>
<tr>
<td>Day 13</td>
<td>16.2 ± 0.5d</td>
<td>14.9 ± 0.4d (24)</td>
</tr>
<tr>
<td>Day 16</td>
<td>15.0 ± 0.5d</td>
<td>471.6 ± 27.1 (17)</td>
</tr>
</tbody>
</table>

bCorpora lutea were collected in Exp. 1. Corpora lutea from two gilts were accidentally lost.
c,dMeans within a column lacking a common superscript differ (P < .05).

Table 2. Characteristics of day 11.5 concepti from vehicle- and testosterone-treated gilts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of concepti (n)</th>
<th>Recovery rate of concepti (n)</th>
<th>Percentage of gilts having 100% recovery rate (n)</th>
<th>Mean conceptus diameter per litter, mm (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>12.8 ± 0.5d (19)</td>
<td>86.6 ± 2.7d (19)</td>
<td>21.1 ± (19)</td>
<td>6.4 ± .7 (17)</td>
</tr>
<tr>
<td>Day 13</td>
<td>15.3 ± 0.6d (17)</td>
<td>94.8 ± 2.8d (17)</td>
<td>47.1 ± (17)</td>
<td>7.2 ± .7 (16)</td>
</tr>
<tr>
<td>Day 16</td>
<td>12.8 ± 0.6d (18)</td>
<td>85.8 ± 2.7d (18)</td>
<td>16.7 ± (18)</td>
<td>5.1 ± .8 (14)</td>
</tr>
</tbody>
</table>

aMean ± SE.
bEffect of treatment group (P = .10).
cGilts having unmeasurable filamentous blastocysts were not included (2, 1, and 4 for vehicle, d 13, and d 16, respectively). In each group one additional gilt had no concepti.
d,eMeans within a column lacking a common superscript differ (P < .05).

Results

Experiment 1. Gilts treated with testosterone from d 13 to estrus had greater (P < .04) numbers of CL than gilts treated with vehicle and tended (P = .07) to have more CL than gilts treated with testosterone from d 16 to estrus. Number of CL in gilts treated with testosterone from d 16 to estrus and those treated with vehicle were not different (P = .77, Table 1).

The number of concepti (P < .01) and percentage of concepti recovered (P < .04) were greater in gilts treated with testosterone from d 13 to estrus than in gilts treated with testosterone from d 16 to estrus or in those treated with vehicle. Gilts treated with testosterone from d 16 to estrus and those receiving vehicle were not different in number (P = .93) or recovery rate (P = .84) of concepti. The proportion of gilts having 100% of their concepti recovered on d 11.5 tended to be greater (P = .10) in gilts treated with testosterone from d 13 to estrus than in the other groups (Table 2).

Mean CL weight (Table 1), mean conceptus diameter (Table 2), and standard deviation of conceptus diameter (estimator of morphological diversity of littermate concepti) were not influenced (P > .10) by treatment. The standard deviations of conceptus diameter per litter were 1.6 ± .2, 1.9 ± .3, and 1.3 ± .3 mm (mean ± SE) for gilts receiving vehicle and those treated with testosterone from d 13 and d 16 to estrus, respectively. The proportion of gilts from which concepti were recovered on d 11.5 was not influenced (P = .99) by treatment and was 19 out of 20 (95%), 17 out of 18 (94.4%), and 18 out of 19 (94.7%) for gilts receiving vehicle or testosterone beginning on d 13 and d 16, respectively.

Experiment 2. Gilts treated with testosterone from d 13 to estrus had more CL (P < .01) than those administered vehicle (Table 1). Similarly, the mean number of total concepti was influenced by the main effect of testosterone treatment: number of total concepti was greater (P < .01) in gilts treated with
testosterone than in those treated with vehicle (Table 3). The mean number of total concepti at the 4- to 8-cell and early blastocyst stages did not differ (P = .80); however, total concepti at these stages were greater (P < .01) than at the hatching stage (main effect of stage of embryonic development, P < .01, Table 3). The proportion of gilts from which concepti were recovered was 100% in all groups.

The number of normal concepti was influenced (P < .05) by the interaction of testosterone treatment and stage of conceptus development (Table 4). The mean number of early blastocysts, classified as normal, was greater (P < .001) in gilts treated with testosterone than in those treated with vehicle. However, the mean number of 4- to 8-cell embryos (P > .73) and hatching blastocysts (P = .13) were not different between these groups. The number of normal concepti in gilts treated with testosterone did not change (P = .30) from the 4- to 8-cell to the early blastocyst stage but decreased (P < .01) by the hatching stage. In contrast, the number of normal concepti in gilts treated with vehicle decreased (P < .02) from the 4- to 8-cell stage to the early blastocyst stage but did not change (P = .41) by the hatching stage (Table 4).

The recovery rate of total concepti was not influenced by the interaction (P = .72) or the main effect of treatment (P = .65); however, it was highly influenced (P < .001) by stage of conceptus development (Table 3). Similarly, recovery rates of normal concepti were not influenced (P = .12) by testosterone treatment but were influenced (P < .001) by stage of conceptus development (Table 4). Although recovery rates of total (P = .97) or normal (P = .20) concepti at the 4 to 8-cell and early blastocyst stages did not differ, recovery rates at these stages were greater (P < .01) than at the hatching blastocyst stage (Table 4).

Experiment 3. Administration of 1 mg of androstenedione per day from d 13 of the estrous cycle to the first day of the following estrus did not influence the number of CL (P = .28) or and the number (P = .63) or recovery rates (P = .69) of concepti on d 11.5 (Table 5). The proportion of gilts from which concepti were recovered were 94.4% and 95.0% for gilts treated with vehicle or androstenedione, respectively.

Discussion

Administration of 1 mg of testosterone on d 17 and 18 of the estrous cycle was previously observed in our laboratory to increase the number of CL and the number of d-11 concepti. Recovery rates of concepti in that experiment were not different between gilts previously treated with testosterone and those receiving vehicle (89.9% and 84.7% respectively, Cárdenas and Pope, 1994). In the present experiments, a longer treatment with testosterone, from d 13 to estrus, consistently increased the number of CL (Exp. 1 and

Table 3. Number and recovery rate of total concepti at different stages of development from vehicle- and testosterone-treated gilts (n)\(^a\)

<table>
<thead>
<tr>
<th>Stage of development</th>
<th>No. of total concepti</th>
<th>Recovery rate of total concepti, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Testosterone</td>
</tr>
<tr>
<td>4 to 8-cell</td>
<td>13.6 ± 0.7 (8)</td>
<td>13.9 ± 0.7 (8)</td>
</tr>
<tr>
<td>Early blastocyst</td>
<td>12.0 ± 0.7 (8)</td>
<td>15.1 ± 0.7 (8)</td>
</tr>
<tr>
<td>Hatching blastocyst</td>
<td>10.5 ± 0.7 (8)</td>
<td>11.9 ± 0.7 (8)</td>
</tr>
<tr>
<td>Main effect</td>
<td>12.5 ± 0.4(^b) (24)</td>
<td>13.6 ± 0.4(^c) (24)</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SE.

Table 4. Number and recovery rate of normal concepti at different stages of development from vehicle- and testosterone-treated gilts (n)\(^a\)

<table>
<thead>
<tr>
<th>Stage of development</th>
<th>No. of normal concepti(^b)</th>
<th>Recovery rate of concepti, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Testosterone</td>
</tr>
<tr>
<td>4 to 8-cell</td>
<td>13.4 ± 0.8(^e) (8)</td>
<td>13.8 ± 0.8(^e) (8)</td>
</tr>
<tr>
<td>Early blastocyst</td>
<td>10.8 ± 0.8(^d) (8)</td>
<td>14.9 ± 0.8(^d) (8)</td>
</tr>
<tr>
<td>Hatching blastocyst</td>
<td>9.9 ± 0.8(^d) (8)</td>
<td>11.5 ± 0.8(^e) (8)</td>
</tr>
<tr>
<td>Main effect</td>
<td>8.5 ± 0.2 (24)</td>
<td>89.6 ± 2.0 (24)</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SE.

\(^b\)Number of normal concepti was influenced (P < .05) by the interaction of treatment and stage.

\(^c\)Means for number of normal concepti lacking a common superscript differ (P < .01).

\(^d\)Means within stage lacking a common superscript differ (P < .001).
2) and also increased recovery rates of d-11.5 concepti (Exp. 1). More gilts treated with testosterone on d 13 had 100% recovery rates than vehicle gilts, and this was in agreement with the actual recovery rate data. The combined effects of testosterone on increasing the number of CL and recovery rate of concepti probably accounted for increasing the number of concepti on d 11.5.

We previously hypothesized that the effects of testosterone on increasing the number of CL might be related to its capacity, as a substrate, to increase follicular concentrations of estradiol. Transiently increasing local concentrations of estradiol was speculated to stimulate follicular development and decrease atresia in the population of large follicles present on d 17 and 18 (Cárdenas and Pope, 1994). Although it is possible that some aspects of this hypothesis apply to the present experiments, it remains to be determined whether testosterone treatment beginning on d 13 exerts other actions independently from, or in addition to, changes in follicular synthesis of estradiol or atresia.

An important finding from the present experiments was the positive effects of exogenous testosterone, when administered from d 13 to estrus, on the proportion of concepti surviving to d 11.5. It seems logical to consider that effects that reduce follicular atresia also augment development of healthy follicles. The development of the porcine oocyte and antral milieu are interrelated (Ainsworth et al., 1980; Hunter and Wiesak, 1990; Xie et al., 1990; Hunter et al., 1992; Koenig and Stormshak, 1993). Additionally, oocytes of younger gilts have been observed to have compromised development compared to those of older gilts (Menino et al., 1989; Archibong et al., 1992; Koenig and Stormshak, 1993). It was of interest to examine whether testosterone spared later-developing follicles. If more of the later-developing follicles ovulated after testosterone treatment, then perhaps the distribution of littermate concepti would reflect a shift to more concepti being at a lesser stage of development. The increase in conceptus survival after treatment with testosterone, however, was not accompanied by significant changes in morphological diversity among littermate concepti on d 11.5. Although not specifically examined in this experiment, either the treatment with testosterone failed to assist ovulation of just the later-developing follicles or testosterone assisted the development of more than one subgroup of follicles. Likewise, exogenous testosterone failed to affect the average weight of resulting CL on d 11.5.

The question of when, before d 11.5, testosterone pretreatment could have helped conceptus survival was examined, in part, in Exp. 2. Although recovery rates of d-4 to d-7 concepti (total or normal) were not significantly influenced by treatment with testosterone, the observations of more normal concepti surviving to d 5.5 in testosterone- than in vehicle-treated gilts suggested that testosterone treatment might have assisted survival before d 5.5. Indeed, the difference in recovery rates at the early blastocyst stage was approximately 11% greater in gilts treated with testosterone than in those receiving vehicle. In Exp. 2, with a smaller sample size than Exp. 1, the pattern of this survival advantage was evident up to the hatching stage of development but lacked statistical significance.

The positive effects of testosterone treatment beginning on d 13 on number of CL and recovery rate of concepti, and the absence of these effects when treatment was initiated on d 16, might suggest that a longer treatment, which included the period of follicular recruitment (d 14 to 16, Foxcroft and Hunter, 1985), was important. In contrast, in a previous investigation, testosterone treatment on d 17 and 18 increased the number of CL (Cárdenas and Pope, 1994). The response to exogenous testosterone might depend on complex and still unknown interaction(s) between dose, duration, and initiation of testosterone treatment.

The lack of effects of 1 mg of androstenedione found in Exp. 3 should not necessarily be considered definitive. Although results of in vitro experiments have demonstrated that granulosa and theca cells secrete more estradiol in the presence of androstenedione than in the presence of testosterone (Tsang et al., 1985), changes in estradiol following androstenedione administration in vivo need verification. Similarly, the 1-mg dose of testosterone was established from dose-response relationships for testosterone (Cárdenas and

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### Table 5. Number of corpora lutea (CL) and characteristics of concepti on day 11 from vehicle- and androstenedione-treated gilts (n)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of CL</th>
<th>Number of concepti</th>
<th>Recovery rate of concepti, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>14.3 ± .5 (18)</td>
<td>12.5 ± .6 (17)</td>
<td>87.2 ± 3.1 (17)</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>15.1 ± .5 (20)</td>
<td>12.9 ± .6 (19)</td>
<td>85.5 ± 3.0 (19)</td>
</tr>
</tbody>
</table>

*Mean ± SE.
Pope, 1994) and these relationships were not established for 1 mg of androstenedione.

In conclusion, testosterone treatment from d 13 to estrus was effective in increasing the number of ovulations and the proportion of concepti surviving to d 11.5. Perhaps testosterone, by improving some aspects of follicular development also improved oocyte quality and augmented embryonic development.

Implications

The ability to favorably influence conceptus survival by administering testosterone during the follicular phase is an interesting biological finding. Increasing the number of concepti surviving up to the period of elongation might represent a potential to increase litter size. In this regard, utilization of small doses of testosterone to increase litter size in gilts seems promising and warrants further investigations.

Literature Cited


