

Effects of Immunization of Gilts Against 17 α -hydroxyprogesterone on Follicular Size Distributions and Follicular Steroid Synthesis

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Story in Brief

The objective of this experiment was to evaluate the effects of immunization of gilts against 17 α -hydroxyprogesterone on follicular size distributions, follicular steroid synthesis, and ovulation rate. Thirty-six crossbred gilts at 147 d of age were immunized against adjuvant (Control; $n = 18$) or 17 α -hydroxyprogesterone (17OHP; $n = 18$). Gilts were given an initial 0.6 mL injection divided between two subcutaneous sites at the base of each ear, followed 4 weeks later by a single booster injection. Estrus was checked twice daily with a boar. At 16 to 17 d following first estrus gilts were sacrificed, reproductive tracts were recovered and uterine weight, uterine length, number of corpora lutea, and number of small (0 to 3 mm), medium (4 to 6 mm), and large (> 7 mm) follicles was determined. Serum binding of 17 α -hydroxyprogesterone in 17OHP was greater ($P < 0.01$) than Control, and increased ($P < 0.01$) with time. Age at puberty was not ($P = 0.28$) affected by treatment and averaged 187.1 ± 0.4 and 183.9 ± 1.7 d for Control and 17OHP, respectively. Serum progesterone during the first 17 d of the estrous cycle was higher ($P = 0.09$) for 17OHP gilts than for Control gilts. Serum estradiol-17 β was not ($P = 0.84$) affected by treatment; however serum progesterone and estradiol-17 β ($P < 0.01$) were affected by time. Uterine weight tended to be increased ($P = 0.12$) in 17OHP vs. Control, whereas mean uterine length was not ($P = 0.82$) different in 17OHP vs. Control (716.8 ± 47.8 vs. 625.3 ± 30.1 g and 214.8 ± 10.7 vs. 211.1 ± 11.2 cm., respectively). Ovulation rate at first estrus was higher ($P = 0.0038$) in 17OHP gilts than Control gilts (15.8 ± 0.6 vs. 13.4 ± 0.4 , respectively). Follicular fluid estradiol-17 β did not ($P = 0.62$) differ between treatments for any follicular size class; however, testosterone tended to be higher ($P = 0.16$) in medium follicles of 17OHP gilts than Control gilts (133.1 ± 22.5 vs. 94.5 ± 13.6 ng/ml, respectively). These data suggest that immunization against 17OHP altered follicular growth and steroid synthesis.

Introduction

The most important factor contributing to the economic efficiency of swine production and the prime measure of reproductive performance is litter size. Ovulation rate and litter size in swine are influenced by a number of factors including breed, age and weight at breeding, and nutritional status at or near the time of breeding. Direct genetic selection for litter size has been of limited success due to low heritability (van der Lende and Schoenmaker, 1990) and decreased number of pigs weaned in the selected line (Johnson et al., 1999).

Cattle and sheep immunized against androgenic steroids had increased ovulation rate (van Look et al., 1978; Streenan et al., 1987). Gilts immunized against androstenedione have increased ovulation rate, but litter size at term was not affected (McKinnie et al., 1988; McKinnie, 1987). Kreider et al. (2001) reported increased ovulation rate and number of live pigs born at term in gilts immunized against 17 α -hydroxyprogesterone; therefore, the objectives of this study were to determine the effects of immunization of 17 α -hydroxyprogesterone on follicular dynamics and ovulation rate.

Experimental Procedures

In this experiment, 36 prepubertal crossbred (Dekalb line 348) gilts at 147 d of age were blocked by litter number and weight, and then randomly assigned to one of two treatment groups: adjuvant only (Control; $n = 18$) or 1.0 mg of 17 α -hydroxyprogesterone-3-CMO:Bovine Serum Albumin (BSA) (17OHP; $n = 18$). Gilts were housed at four animals/pen in 1.5 x 4.0 m slotted floor pens in a naturally ventilated curtain-sided finishing building, and were fed 1.8 kg/d of a ration formulated to meet, or exceed NRC (1998) recommendations. Immunization procedures used were similar to those previously described by Kreider et al. (2001) with the exception that

Freund's incomplete adjuvant was used instead of light mineral oil. One milligram of 17 α -hydroxyprogesterone-3-CMO:BSA was dissolved in 0.3 mL of 5% DEAE dextran (5% w/v in 0.45% saline) and emulsified with an equal volume of Freund's incomplete adjuvant. Gilts received an initial 0.6 mL injection divided equally between two subcutaneous sites in the loose skin at the base of each ear, and then four weeks later a single 0.3 mL booster injection.

After the booster immunization, gilts were monitored twice daily with a boar to determine estrus. At 16 to 17 d after the first detected estrus, gilts were sacrificed, and reproductive tracts were recovered. Uterine size (length and weight of trimmed uterus), number of corpora lutea, and number of follicles (0 to 3 mm, 4 to 6 mm, and > 7 mm) were determined, and follicular fluid for each size class was aspirated from follicles into a 1 mL syringe using a 22-gauge needle (pooled within size class within gilt). Follicular fluid was stored frozen at -20°C until assayed for estradiol 17- β and testosterone. Blood samples were collected via anterior vena cava puncture immediately prior to first immunization, at the booster immunization, and at d 0, 4, 8, 12, and 16 of the estrous cycle. Blood was allowed to clot, centrifuged for 20 min, serum was harvested, and serum samples were stored at -20°C until assay. Serum was analyzed for progesterone, testosterone, and estradiol-17 β by RIA. Serum binding of 17 α -hydroxyprogesterone was determined by measuring the percent of counts bound by a 1:100 dilution of serum when approximately 20,000 dpm of the appropriate tritium labeled antigen was added (Scaramuzzi et al., 1975).

Differences between the control and treatment (17OHP) groups were determined by analysis of variance. Data for reproductive variables were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Hormone and antibody binding were analyzed as split plot analyses using the repeated measures option of GLM. Four gilts in the control group and three gilts in the 17OHP group did not show estrus and were eliminated from the study.

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Results and Discussion

Results for reproductive variables are summarized in Table 1. Immunization of gilts against the androgenic steroid precursor, 17α -hydroxyprogesterone, did not ($P = 0.28$) influence age at puberty. For the control group, age at puberty averaged 187.1 ± 2.4 d ($n = 14$), whereas the 17OHP group averaged 183.9 ± 1.7 d ($n = 15$). Uterine length was not ($P = 0.82$) affected by immunization against 17α -hydroxyprogesterone, but uterine weight tended to increase ($P = 0.12$) with the immunization against 17α -hydroxyprogesterone.

Immunization against 17α -hydroxyprogesterone increased ($P = 0.0038$) ovulation rate at first estrus, as determined by the number of corpora lutea present on the ovaries at slaughter. Mean ovulation rate for the control group was 13.4 ± 0.4 vs. 15.8 ± 0.6 for the 17OHP treatment group. Total number of follicles was not ($P = 0.44$) different overall, or in the three sizes of follicles, small (0 to 3 mm), medium (4 to 6 mm) and large (> 7 mm). Number of small (0 to 3 mm) follicles averaged 47.6 ± 8.0 for the control group and 58.5 ± 12.2 for the 17OHP treatment group ($P = 0.47$). The number of medium follicles (4 to 6 mm) averaged 9.0 ± 1.9 for the control group and was numerically greater than the 6.0 ± 1.5 for the 17OHP group, but groups were not statistically different ($P = 0.22$). In contrast the number of large follicles (> 7 mm) was numerically greater in 17OHP gilts than in controls, but not statistically higher ($P = 0.32$).

Concentrations of steroids in follicular fluid are presented in Table 2. Follicular fluid concentrations of testosterone in small follicles and large follicles did not differ ($P = 0.31$, $P = 0.37$, respectively) between the 17OHP and control gilts. Testosterone concentrations in medium follicles were numerically different ($P = 0.16$) between the 17OHP and the control gilts. Follicular fluid concentrations of estradiol- 17β were not ($P > 0.6$) different for any size class between 17OHP and control gilts.

Serum binding of 17α -hydroxyprogesterone is illustrated in Figure 1. The serum binding response in the 17OHP treatment group was higher ($P = 0.004$), than the control group and increased with time ($P = 0.001$), indicating that a significant immune response was elicited by the antigen-adjuvant preparation used in this study.

Serum progesterone and estradiol responses during the estrous cycle are shown in Figures 2a and 2b. Serum progesterone during d 0 to 16 of the estrous cycle tended to be greater for gilts immunized against 17α -hydroxyprogesterone ($P = 0.09$) and was affected ($P < 0.0001$) by time. In contrast, concentration of serum estradiol- 17β during d 0 to 16 of the estrous cycle was not affected ($P = 0.84$) by treatment, but was affected by time ($P = 0.0002$).

Results of this experiment indicated that immunization of gilts

against 17α -hydroxyprogesterone increased ovulation rate at first estrus. This increase in ovulation rate is similar to that observed at the second estrus by Kreider et al. (2001) in gilts immunized against 17α -hydroxyprogesterone.

The increase in serum binding of 17α -hydroxyprogesterone over time indicates that the antigen-adjuvant preparation and immunization procedure were effective in eliciting an immune response against 17α -hydroxyprogesterone. In contrast to our previous study (Kreider et al., 2001), serum progesterone was greater in gilts immunized against 17α -hydroxyprogesterone throughout the estrous cycle vs. controls. The increase in progesterone was likely due to an increase in the number of corpora lutea and/or luteinized follicles. Follicular size class distributions were numerically different between treatments, but were not statistically altered. Follicular testosterone concentrations in 17OHP gilts tended to be higher in medium sized follicles versus controls.

Implications

The results of this study indicate that immunization of gilts against 17α -hydroxyprogesterone can effectively increase ovulation rate, and suggest effects on follicular size distributions and follicular steroid concentrations. Further research is needed to substantiate these results.

Literature Cited

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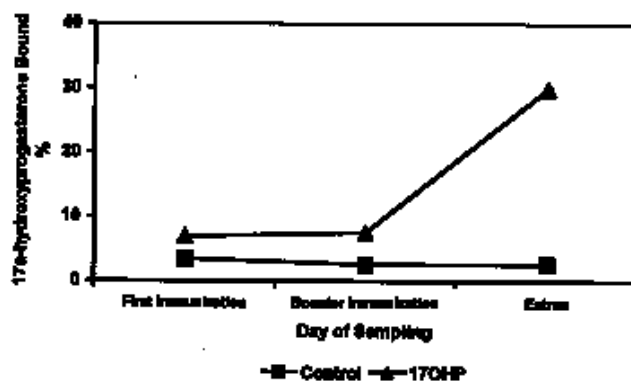


Fig. 1. Serum binding of 17α -hydroxyprogesterone.

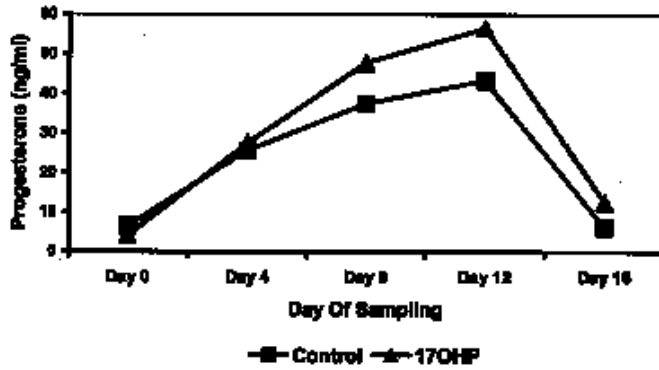


Fig. 2a. Serum progesterone concentration.

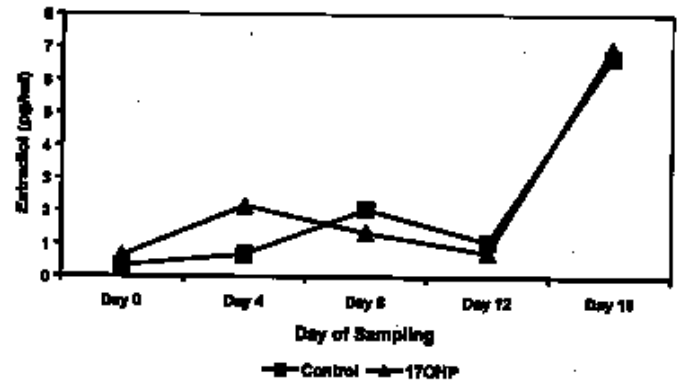


Fig. 2b. Serum Estradiol Concentration.

Table 1. Effects of immunization of gilts against 17 α -hydroxyprogesterone (17OHP) on reproductive variables and ovarian structures at day 16 or 17 of the estrous cycle.

Item	Treatment		Probability
	Control	17OHP	
Number	14	15	
Age at puberty, d	187.1 \pm 2.4	183.9 \pm 1.7	0.28
Uterine length, cm	211.1 \pm 11.2	214.8 \pm 10.7	0.82
Uterine weight, g	625.3 \pm 30.1	716.8 \pm 47.8	0.12
Corpora lutea, n	13.4 \pm 0.4	15.8 \pm 0.6	0.0038
Small follicles, n	47.6 \pm 8.0	58.5 \pm 12.2	0.47
Medium follicles, n	9.0 \pm 1.9	6.0 \pm 1.5	0.22
Large follicles, n	3.1 \pm 1.2	5.1 \pm 1.6	0.32
Total follicles, n	59.6 \pm 7.0	69.5 \pm 10.2	0.44

Table 2. Effects of immunization of gilts against 17 α -hydroxyprogesterone on concentrations of testosterone and estradiol-17 β in follicular fluid.

Item	Follicle size	Treatment		Probability
		Control	17OHP	
Testosterone ng/ml	1 to 3 mm	33.4 \pm 7.3	44.8 \pm 8.2	0.31
	4 to 6 mm	94.5 \pm 13.6	133.1 \pm 22.5	0.16
	> 7 mm	138.9 \pm 26.7	111.5 \pm 16.0	0.37
Estradiol ng/ml	1 to 3 mm	25.3 \pm 8.4	29.6 \pm 6.3	> 0.6
	4 to 6 mm	244.2 \pm 39.5	275.5 \pm 56.3	> 0.6
	> 7 mm	396.2 \pm 55.6	346.6 \pm 78.2	> 0.6