PUBERTY INDUCTION IN YOUNG GILTS: OVARIAN, UTERINE, AND PREGNANCY RESPONSES

J. Guo, D. M. Grieger, and D. L. Davis

Summary

The age of gilts when their first litter is produced affects reproductive efficiency and the applications of biotechnologies such as in vitro fertilization and genetic engineering. Therefore, we evaluated the effect of age on response to puberty induction in gilts. Gilts were injected with PG600® followed 96 h later with human chorionic gonadotropin to induce follicular growth and ovulation, respectively. In the first experiment, 84-, 104-, 124-, 144-, and 164-d-old gilts were used. For treated gilts, uterine weight, uterine length, number of corpora lutea (CL), peripheral progesterone (P_4), and estradiol (E_2) increased ($P < .05$) linearly with age. Uterine luminal prostaglandins (PGs) PGE and PGF decreased for gilts treated at 124 d of age or older. The second experiment evaluated pregnancy success for gilts induced to ovulate at 116 vs 151 d of age. The effects of induction of two consecutive estrous cycles also were evaluated. Two of seven (28.6%) and four of nine (44.4%) gilts first treated when 116 and 151 d old but none of seven gilts treated at both 96 and 116 d of age were pregnant 60 d postinsemination. Results indicated that induction of a prior cycle did not improve pregnancy rates. However, some gilts in this population maintained pregnancies to 60 d when induced to ovulate and inseminated at 120 d of age.

(Key Words: Puberty, Induction, Gonadotropin Treatment, Gilt.)

Introduction

Reproductive ability in gilts develops over a prolonged period. Spontaneous puberty is neither the beginning nor the end of the sequence that begins before birth and extends through the postpubertal period. Prepubertal gilts can be induced to ovulate by around 100 d of age by treatment with pregnant mare's serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG). Eggs released at induced puberty can be fertilized and begin development, but pregnancies generally are not maintained in very young gilts. Also, gilts induced to ovulate at young ages do not continue to cycle. Furthermore, gilts mated at spontaneous puberty may have reduced litter size compared to gilts mated at later estruses, and this can be attributed to fewer ovulations at the pubertal estrus and increased embryonic mortality following mating at puberty.

Therefore, development of reproductive ability in gilts includes: (1) attainment of the ability to ovulate in response to exogenous gonadotropins (by 100 d), (2) attainment of the ability to maintain pregnancy and to continue estrous cycles (by 150 d), and (3) increased ovulation rate and embryonic survival rates (through the second or third estrous cycle). An understanding of the multiple and interrelated maturations resulting in fertility in postpubertal gilts is important for decreasing the age at first reproduction to achieve more efficient pork production and for utilizing gonadotropin-treated gilts for embryo donors or recipients in the application of in vitro fertilization, transgenics, nuclear transplantation, and other biotechnologies. Our objectives were to determine the maturation of certain uterine responses relative to the attainment of fertility.

Procedures

Experiment 1. Crossbred gilts (PIC lines C15×326) were treated with gonadotropins (PG600®; 400 IU pregnant mare's serum gonadotropin-200 IU human chorionic gonadotropin, Intervet America Inc.) at 84, 104, 124, 144, or 164 d of age and average weights of 95.3, 115.5, 152.0, 184.8, and 225.9 lb, respectively, and received hCG (500 IU) 96 h later. Control gilts were included at 104 (117.9 lb) and 144 d (189.9 lb) and received only saline injections. Blood was collected from the jugular vein before (d 0) and after (d 2, 3, 4, 8, and 16) the first injection, and serum was harvested for assays of progesterone (P_4) and estradiol (E_2). Uteri were removed by hysterectomy on d 16 after initial gonadotropin treatment. Before removing the uterus, blood was collected from a uterine vein, and plasma was harvested for assay of prostaglandins (PGs). The number of corpora lutea (CL) was determined to estimate the number of ovulations, and the uterus was weighed. The length of one uterine horn was measured after trimming it free of the broad ligament, and the other horn was flushed with 20 ml of saline (.85% NaCl). Flushings were collected for PG assays.

Experiment 2. Twenty four crossbred gilts (PIC lines C15×326) were assigned to one of three treatments. Seven gilts were injected intramuscularly with gonadotropin at 96 d of age (133.1 lb) and with hCG (500 IU) 96 h later to induce ovulation. At 116 d of age (the predicted time of luteolysis), a second gonadotropin treatment was administered followed 96 h later by hCG. Another seven gilts first were administered gonadotropin at 116 d of age (143.9 lb), and 10 other gilts received gonadotropin at 151 d of age (213.6 lb). Those latter two groups of gilts also received hCG 96 h after gonadotropin treatment. Gilts were housed in outdoor pens and provided with a shed, water, and ad libitum access to feed. Before gonadotropin treatment, on the day of hCG injection, and on d 13 and 21 after hCG treatment, peripheral blood was collected for E_2 and P_4 determinations. Two inseminations were given to all gilts, on the day of hCG treatment and 24 h later. Gilts given gonadotropin at both 96 and 116 d of age were inseminated only after the second hCG injection. Jugular blood was collected 21 d after hCG for assay of P4. Approximately 60 d after hCG, surgeries were performed on gilts that had elevated concentrations of P_4 at 21 d after hCG, and fetuses were collected.

Results and Discussion

Experiment 1. Ovaries of 104-d-old control gilts contained follicles ($\leq$ 3mm), but no CL were present. In the 144-d-old control group, recently regressed or active CL were found in ovaries in two gilts, indicating that they had reached puberty, but no indication of ovulation was seen in the other two control gilts whose ovaries contained only small follicles. In comparison, all gonadotropin-treated gilts had CL (Table 1). For gonadotropin-treated gilts, uterine weight and length exhibited a positive linear relationship with age ($P<.05$). The ovarian response to gonadotropins was reflected in the numbers of CL, which varied from one to 68 and increased linearly ($P<.05$) with age.

The peak of E_2 occurred on d 2 or 3 after gonadotropin. Concentrations of E_2 on d 0 (before treatment) and on d 4 were greater ($P<.05$) for 164-day-old gilts. For gonadotropin-treated gilts, P_4 increased from d 8 to 16 after gonadotropin (d 4 to 12 after hCG). Concentrations of P_4 on d 4 increased ($P<.05$) quadratically with age, and P_4 on d 8 ($P=.06$) and d 16 ($P<.05$) increased linearly with age. Concentrations of P_4 in serum on d 8 and 16 were correlated ($P<.01$) positively with number of CL, and P_4 concentrations on d 4, 8, and 16 tended ($P<.1$) to be correlated positively with uterine weight.

Compared to controls of the same ages, gilts treated at 104- and 144-d of age had heavier (424.0 ± 39.3 vs 99.8 ± 48.1 g) and longer (467.6 ± 21.0 vs 308.0 ± 25.7 mm) ($P<.01$) uteri (Table 1), more ($P<.05$) P_4 on d 8 (8.4 ± 1.7 vs $.4\pm2.1$ ng/ml) and 16 (22.2 ± 5.5 vs $.3\pm6.7$ ng/ml), and more

E_2 on d 2 (16.7±5.3 vs 1.2± 6.5 pg/ml) ($P<.1$) and d 4 (4.8±.6 vs 1.9±.8 pg/ml) ($P<.05$).

For gonadotropin-treated gilts, PGE recovered from the uterine lumen was high for 84- to 104-d-old gilts and then decreased (Table 2), showing a quadratic effect of age ($P=.05$). Lower concentrations of PGs were recovered from the uterine lumens of gilts 124 d or older at treatment. Uterine luminal PGs/g uterus for 144- and 164-d-old-gilts was approximately 10% that observed for 104-d-old gilts. Concentrations of PGF in uterine venous plasma tended to have a linear relationship with age ($P<.10$) (Table 3). Interestingly, the amount of PGF exceeded that of PGE in the uterine lumen, but the ranking of the two PGs was reversed in the uterine vein.

Experiment 2. Five of seven gilts first induced to ovulate at 96 d of age had elevated (above 2 ng/ml) concentrations of P_4 4 d after the first hCG treatment. When these gilts were retreated with gonadotropin and hCG and inseminated, six of them had increased P_4 13 d after the second hCG injection. Progesterone concentrations for one gilt in this treatment were elevated (4.2 ng/ml) 21 d after AI, but she was not pregnant at laparotomy on d 60.

All gilts initially treated at 116 d of age had increased P_4 13 d after hCG injection, and three gilts maintained concentrations of P_4 greater than 2 ng/ml at 21 days after AI, which was indicative of pregnancy. All gilts first given gonadotropin at 151 d of age had P_4 concentrations greater than 2 ng/ml 13 days after hCG injection. Six of these gilts maintained greater than 2 ng/ml P_4 21 d after AI. Examination of uteri and ovaries for gilts that had elevated P_4 21 days after AI revealed that two of seven gilts first treated at 116 d of age and four of nine gilts treated at 151 d of age maintained pregnancies. The two gilts treated at 116 d had five and nine fetuses, representing 71% and 60% of CL, respectively. The four gilts 151 d old at treatment had four to 12 fetuses (50 to 91% of CL). Fetal weights and lengths were within the range expected for approximately d 60 of gestation.

Gilts induced to ovulate around 120 d of age in previous studies did not maintain pregnancy unless exogenous hormones were given. However some gilts in the population used in our experiment were able to establish pregnancies when ovulation was induced at 124 d of age (Exp. 2). Two gilts in the 144-d-old control group and three gilts in the 164-d-old treated group had CA and/or CL before treatment, indicating that they had already attained puberty. Therefore, gilts in these latter two age groups that had not ovulated before treatment may be considered peri-pubertal, and our gilts apparently matured earlier than those reported in earlier studies. Considering these observations, the responses of 124-d-old gilts in our experiments may be important for supporting pregnancy. Prostaglandin content of the uterine lumen, expressed per g uterine weight, dropped markedly by 124 d of age. The effect on pig embryos of residing in an environment with high PG concentrations has not been determined, but studies in postpartum cows suggest that a high PGF concentration in the uterine lumen is toxic to embryos.

Knowledge of the development of reproductive ability in gilts is important to reduce age at first reproduction and to apply biotechnologies utilizing oocytes and embryos. Our data describe the development of uterine PGE and PGF secretions in response to gonadotropin treatment. The data indicate that secretion of PGs into the uterine lumen of gonadotropin-treated gilts declines at the time when pregnancies can be maintained. An understanding of the maturation events leading to decreased PGs and increased proteins in the uterine lumen as gilts are induced to ovulate nearer the time of spontaneous puberty may be useful for reducing the age at first reproduction and, thereby, improving the efficiency of pork production. The relationship of this change to the ability of gilts to maintain pregnancy should be the subject of future investigations.

Table 1. CL, Uterine Weight (UTWT), and Uterine Length (UTLG) on Day 16 after PG600/Saline Treatment

Age[a], day	No. of Gilts	CL[b]	UTWT(g) [b]	UTLG(mm) [b]
Controls[c]				
104	4	–	75.6±55.6	285.5±29.7
144[d]	2	–	124.0±78.6	330.5±42.0
Gonadotropin-Treated				
84	4	2.8±6.5	122.1±60.3	306.3±37.0
104	4	11.5±6.5	268.6±60.3	365.8±37.0
124	4	17.8±6.5	500.4±60.3	606.3±37.0
144	4	18.8±6.5	579.5±60.3	569.5±37.0
164[e]	2	45.5±9.1	742.6±85.2	585.0±52.0

[a]Age at PG600 injection.
[b]Linear effect ($P<.05$) of age in gonadotropin-treated gilts.
[c]UTWT and UTLG are less ($P<.05$) than those of gonadotropin-treated gilts of the same ages.
[d]Two controls had reached puberty and are not included.
[e]Three gilts had reached puberty before treatment and are not included.

Table 2. Total Prostaglandin (pg/g) Recovered in Uterine Flushings per Gram Uterine Weight on Day 16 after PG600 Treatment

Age, d	No. of Gilts	PGE[a]	PGF[b]
Controls			
104	4	17.5±7.9	79.7±48.9
144	2	5.0±11.2	43.4±69.2
Gonadotropin-Treated			
84	4	11.2±15.4	83.1±125.7
104	4	36.0±15.4	281.5±125.7
124	4	.8±15.4	9.6±125.7
144	4	1.7±15.4	17.4±125.7
164	2	2.7±21.7	39.6±177.8

[a]Quadratic effect ($P=.05$) of age in gonadotropin-treated gilts.
[b]Quadratic effect ($P<.1$) of age in gonadotropin-treated gilts.

Table 3. Prostaglandin (ng/ml) Concentrations in Uterine Venous Plasma on Day 16 after PG600 Treatment

Age, d	No. of Gilts	PGE	PGF[a]
Controls			
104	4	6.6±1.5	.4±.2
144	2	6.3±2.2	1.4±.3
Gonadotropin-Treated			
84	4	9.4±2.1	1.3±.3
104	4	6.2±2.1	.5±.3
124	4	2.2±2.1	.3±.3
144	4	7.6±2.1	.5±.3
164	2	6.9±3.0	.4±.4

[a]Linear effect ($P<.1$) of age in gonadotropin-treated gilts.