Failure of Gonadotropin Therapy to Induce Estrus in Gilts Treated with a GnRH Analog to Suppress Ovarian Activity

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ABSTRACT

A study was conducted to determine whether treatment with deslorelin, a gonadotropin-releasing hormone (GnRH) analog, produced anestrus in gilts and to test the ability of exogenous gonadotropin treatment with a combination of 400 IU equine chorionic gonadotropin and 200 IU human chorionic gonadotropin to induce estrus in GnRH analog-treated gilts. Twelve days after the third estrus, 11 gilts each received a full SC implant containing 2.1 mg deslorelin, a half implant containing 1.05 mg deslorelin, or a sham implant. The implants remained in place for the duration of the study. Approximately 23 days after placement of implants, GnRH analog-treated gilts that had not returned to estrus (n = 18) received IM injections of either 400 IU equine chorionic gonadotropin and 200 IU human chorionic gonadotropin or deionized water. The percentage of gilts with 3- to 4-mm follicles 4 days after implant insertion was significantly ($P < .05$) higher in controls than in gilts receiving a deslorelin implant. The percentage of deslorelin-implanted gilts that displayed a normal estrous cycle was significantly ($P < .05$) lower than in individuals given a sham implant. There was no significant difference in the percentage of implanted-gilts that displayed estrus within 10 days after injection of either 400 IU equine chorionic gonadotropin and 200 IU human chorionic gonadotropin or deionized water. Treatment with a product containing a combination of 400 IU equine chorionic gonadotropin and 200 IU human chorionic gonadotropin failed to induce estrus in gilts caused to be in anestrus by implanting the GnRH analog deslorelin.

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) is secreted by the hypophysiotropic neurons of the hypothalamus. The decapeptide induces synthesis and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH)

from gonadotropes located in the anterior pituitary gland.[1] Since the initial discovery of GnRH as the hypothalamic factor controlling gonadotropin release, numerous GnRH agonists and analogs have been developed and studied for their pro-fertility potential.[2,3]

Paradoxically, suppressive effects of potent GnRH analogs on reproduction have been reported. For example, chronic treatment of cows with a GnRH analog resulted in an initial stimulation of gonadotropin release, which was soon followed by suppressed secretion, and ultimately, inhibition of ovarian follicular development.[4] The mechanism by which GnRH analogs cause reduced gonadotropin secretion and abated ovarian function presumably involves a desensitization or down regulation of GnRH receptors on the anterior pituitary gland.

Miller et al[5] reported that treatment of gilts during the luteal phase of the estrous cycle with a potent GnRH agonist caused suppression of ovarian activity that was reversed 28 days later by exogenous gonadotropin administration. Reproductive efficiency in swine breeding herds could be enhanced by effective methods of synchronizing estrus in replacement gilts. In theory, gilts could be treated with a GnRH analog to cause a state of anestrus and then synchronized estrus could be induced by administration of gonadotropin. The objectives of this study were to determine whether a GnRH analog would produce a state of anestrus in gilts and to test the ability of exogenous gonadotropin treatment to induce estrus in GnRH analog-treated gilts. Commercially available GnRH analog (Ovuplant; Fort Dodge Animal Health) and gonadotropin (P.G. 600; Intervet America) products were employed.

MATERIALS AND METHODS

Preparation of Subjects

The study was conducted at the Virginia Tech-Tidewater Agricultural Research and Extension Center in Suffolk, VA, and the study protocol was reviewed and approved by the Institutional Animal Care Committee. Prepubertal Yorkshire × Landrace gilts (n = 42) approximately 180 days of age were given an IM injection of P.G. 600 containing 400 IU equine chorionic gonadotropin (eCG) and 200 IU human chorionic gonadotropin (hCG). Gilts were checked for estrus once daily beginning at the time of P.G. 600 administration. Estrus was defined as the period during which gilts displayed a lordosis response in the presence of a mature boar. Within 7 days after the injection (4.3 ± 0.1 days), 83% (35/42) of the gilts displayed estrus. Of these 35, 97.1% subsequently displayed a second estrus and 94.3% a third estrus at approximately 21-day intervals. Gilts that had displayed three periods of estrus (i.e., two estrous cycles) were selected to participate in the study.

On Day 12 after the third estrus, 11 gilts each received in the base of an ear a full SC implant containing 2.1 mg deslorelin, a half implant containing 1.05 mg deslorelin, or a a sham implant. The deslorelin implants remained in place for the duration of the study. Gilts returning to estrus between Days 19 and 22 of the third estrous cycle were considered to have had a cycle of normal duration.

Approximately 23 days after insertion of implants (Day 35.4 ± 0.5), deslorin-treated gilts that had not returned to estrus (n = 18) received IM injections of either P.G. 600 (n = 9) or deionized water (n = 9). Of the nine gilts that had received the full deslorelin implant, four were injected with P.G. 600 and five received deionized water. Of the nine gilts that had received the half implant, five were injected with P.G. 600 and four received deionized water.

Ultrasonography

Transrectal ultrasonography of the ovaries was performed 4 days after implant insertion using a real-time, B-mode ultrasound machine (Aloka SSD-500; Aloka) equipped with a linear-array 5-MHz transducer designed for intrarectal placement. The gilts were restrained in standing position inside a portable scale. The ultrasound transducer was

modified by taping a half-moon polyvinyl chloride pipe along the dorsal part of the probe to control manipulation of the transducer in the rectum. The modified transducer was well lubricated with corn oil before insertion into the rectum and was gently passed along the rectal floor to avoid fecal material resistance and to establish good contact between the transducer and the rectal mucosa.

Blood Samples and Radioimmunoassay

Beginning at implant insertion and continuing until estrus or injection of either P.G. 600 or deionized water, blood samples were collected via jugular venipuncture at 4-day intervals. Samples were allowed to clot overnight at 4˚C. Serum was harvested following centrifugation and stored at –20˚C until assayed. Serum progesterone concentrations were determined using a validated radioimmunoassay method described for swine by Tarraf and Knight.[6] The intraassay and interassay coefficients of variation were 4.6% and 8.9%, respectively. Assay sensitivity was 0.02 ng/mL serum.

Statistical Analysis

The interestrus interval was defined as the period between the onset of two subsequent periods of standing estrus. The percentage of gilts displaying estrus after treatment and the percentage of gilts with 3- to 4-mm follicles 4 days after treatment (Day 16 of estrous cycle) were compared using chi-square analysis. The interestrus interval and serum progesterone concentrations were analyzed by analysis of variance using the GLM procedure of SAS (SAS Institute).

RESULTS

Insertion of implants containing the GnRH analog deslorin at Day 12 of the estrous cycle resulted in suppression of follicular growth. The percentage of gilts with 3- to 4-mm follicles 4 days after implant insertion was significantly ($P < .05$) higher in controls than in gilts receiving a full or half implant (Table 1). The percentage of implanted gilts that displayed a normal estrous cycle was significantly ($P < .05$) lower than for sham-implanted individuals (Table 1). There was no significant effect of treatment ($P > .05$) on the interestrus interval for gilts displaying estrus after implant insertion (Table 1).

There were no significant ($P > .05$) differences between groups in the concentrations of progesterone at implant insertion on Day 12 or at Day 16 of the estrous cycle. In deslorin-treated gilts, concentrations of progesterone in serum remained low until the time of P.G. 600 or deionized water administration at approximately Day 35 (Table 1). There was no significant ($P > .05$) difference in the percentage of implanted-gilts that displayed estrus within 10 days after injection of either P.G. 600 or deionized water (Table 2).

DISCUSSION

Implants containing the potent GnRH analog deslorelin were successful at producing anestrus in gilts by inhibiting follicular growth. Four days after insertion of implants containing either 1.05 or 2.1 mg deslorelin, suppressed follicular development was observed ultrasonographically. The lack of estrous cyclicity was confirmed by the observation of low progesterone concentrations in serum for an extended period after implant insertion. The mechanism mediating this prolonged anestrus probably involved suppression of gonadotropin secretion and ultimately an inhibition of follicular development. Although serum concentrations of gonadotropins were not determined in this study, previous work from the authors' laboratory demonstrated that treatment of male swine with deslorelin implants decreased secretion of LH, testosterone, and estradiol.[7]

Treatment with the GnRH analog deslorelin caused a desensitization of the female bovine pituitary gland to GnRH.[8] Treatment with deslorelin for 3 days resulted in a prolonged period of anestrus in postpartum dairy cows implanted within 3 days of calving.[9] As postulated in cattle, the mechanism by which deslorelin reduces LH secretion in pigs could involve a decrease in gonadotrope concentration of GnRH recep-

Table 1. Reproductive Characteristics of Gilts Given a Full (2.1 mg) or Half (1.05 mg) GnRH Analog (deslorelin) or Placebo Implant on Day 12 of the Estrous Cycle

Variable	Sham (n = 11)	Full Implant (n = 11)	Half Implant (n = 11)
Percentage of gilts with 3- to 4-mm follicles 4 days after implant	100[a]	18.2[b]	18.2[b]
Percentage of gilts displaying 19- to 22-day estrous cycle	100[a]	18.2[b]	18.2[b]
Interestrus interval for gilts displaying 19 to 22 d estrous cycle (days)[c]	20.8 ± 1.0	20.0 ± 0.0	20.0 ± 1.4
Progesterone concentrations (ng/mL serum)[c]			
Day 12	44.3 ± 9.5	38.5 ± 6.7	40.6 ± 8.2
Day 16	2.1 ± 2.4	12.0 ± 18.7	1.9 ± 1.3
Day 20	—	0.6 ± 0.9	0.2 ± 0.3
Day 24	—	0.4 ± 0.5	0.2 ± 0.3
Day 28	—	0.2 ± 0.2	0.2 ± 0.4
Day 32	—	0.1 ± 0.1	0.1 ± 0.1
Day 36	—	0.03 ± 0.03	0.1 ± 0.1

[a,b]Values within rows with different superscripts are significantly different ($P < .05$).

[c]Values are mean ± SD.

Table 2. GnRH Analog-Implanted Gilts in Estrus Within 10 Days after Treatment with P.G. 600 or Deionized Water

Treatment	Half Implant (1.05 mg deslorelin)		Full Implant (2.1 mg deslorelin)	
	No. of Gilts	Gilts in Estrus (%)	No. of Gilts	Gilts in Estrus (%)
P.G. 600[a]	5	0	4	25
Deionized water	4	0	5	0

[a]P.G. 600 = 400 IU equine chorionic gonadotropin and 200 IU human chorionic gonadotropin.

tors and GnRH receptor mRNA.[10] The effect of deslorelin on LH β-subunit synthesis could be another mechanism mediating reduced secretion of LH after continuous exposure to GnRH in pigs. When deslorelin implants were inserted on Day 7 postpartum in diary cows, ovulation of the follicles present was observed in 62% of the animals.[11] However, a subsequent block in follicular growth and lower plasma progesterone and estradiol concentrations were also observed for approximately 50 days, suggestive of down regulation of GnRH receptors.

The use of deslorelin in combination with 400 IU eCG and 200 IU hCG for synchronizing estrus in gilts would appear to have little value. In the study described here, treatment with eCG and hCG (P.G. 600) failed to produce estrus in gilts in anestrus from the use of implants containing deslorelin. In accordance with these findings, gonadotropin therapy failed to induce follicle growth in gilts with low gonadotropin levels caused by hypophysectomy, hypophysial stalk-transection, or immunization against GnRH.[12–14]

Miller et al[5] gave an IM injection of 7.5 mg of the GnRH agonist triptorelin to gilts (Decapeptyl, Ferring) between Days 7 and 14 of the estrous cycle, resulting in a suppression of LH and FSH secretion and a block of follicular development. In contrast with results of the present study, however, follicle growth in triptorelin-treated gilts was stimulated by an injection of 1,500 IU eCG given 28 days later. Gilts were slaughtered to recover ovaries 72 hours after eCG treatment, so it is uncertain whether or not

the gilts would have eventually displayed behavioral estrus and ovulated.

The dichotomous results between the current study and the report of Miller et al[5] are not easily explained, but it could be due to the different compounds used to cause a state of anestrus. Perhaps the implants used in the report described here as well as the surgical manipulations and treatments previously employed caused such a severe suppression of endogenous LH and/or FSH secretion that treatment with exogenous gonadotropins could not drive follicular development.[12–14] In support of this notion, Kraeling et al[15] reported that eCG stimulated follicular growth and ovulation in hypophysial stalk-transected gilts receiving pulsatile administration of GnRH but not in hypophysial stalk-transected females receiving saline. Alternatively, the doses of gonadotropins used in the current study (400 IU eCG and 200 IU hCG) may have been an insufficient stimulus. Miller et al[5] administered 1,500 IU eCG.

The GnRH analog deslorelin produced a state of anestrus in gilts, presumably due to a desensitization of pituitary GnRH receptors, a subsequent suppression of gonadotropin secretion, and ultimately, an inhibition of follicular growth. Treatment with 400 IU eCG and 200 IU hCG failed to cause estrus in gilts that were in anestrus by treatment with the GnRH analog.

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