OVULATION AND EMBRYONIC SURVIVAL IN PUBERTAL GILTS TREATED WITH GONADOTROPIN RELEASING HORMONE¹,²

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ABSTRACT

An experiment was conducted to evaluate the effect of exogenous gonadotropin releasing hormone (GnRH) on ovulation and embryonic survival in pubertal gilts. Gilts were assigned in replicates to a control (n = 10) and treatment (n = 10) group. Treatment consisted of an iv injection of 200 µg of GnRH immediately after initial mating on the first day of detected estrus. Control gilts were similarly injected with physiological saline. Blood samples were collected from the anterior vena cava immediately prior to injection, thereafter at 15-min intervals for 90 min, and subsequently, before slaughter on d 30 of gestation. Serum samples were analyzed for luteinizing hormone (LH) and progesterone by radioimmunoassay. Treatment with GnRH increased the quantity of LH released (P<.05), with highest serum concentrations (ng/ml, X ± SE) of gonadotropin in treated gilts (17.3 ± 3.5) occurring at 75 min post-injection. In control gilts, serum concentrations of LH were not affected by injection of saline. Mean number of ovulations in treated gilts was also greater (P<.05) than that of control animals (14.5 ± .7 vs 12.1 ± .6). However, treatment with GnRH did not enhance the number of attached conceptuses (normal and degenerating) present (treated, 10.9 ± .9 vs control, 10.5 ± .7) nor the percentage of viable fetuses (treated, 74.7 ± 6.9 vs control, 83.5 ± 5.0%) on d 30 of gestation. Although GnRH increased ovulation rate, mean weight of corpora lutea of treated and control gilts did not differ (402.8 ± 16.3 vs 389.5 ± 11.3 mg, respectively). Similarly, serum concentrations of progesterone (ng/ml) on d 30 of gestation did not differ between treated and control animals (25.0 ± 2.6 vs 22.1 ± 2.9, respectively). These data indicate that a GnRH-induced increase in number of ovulations in the pubertal gilt is not accompanied by an increase in embryonic survival or litter size at 30 d of gestation. (Key Words: Gonadotropin Releasing Hormone, LH, Ovulation, Embryo Mortality, Gilts.)

Introduction

It has been established that embryonic mortality in gilts bred at pubertal estrus is greater than that of gilts mated at third estrus (Warnick et al., 1951; MacPherson et al., 1977; Archibong et al., 1987). The reason for this disparity in embryonic survival is not known with certainty, but cannot be attributed to a hostile uterine environment in the pubertal gilt (Archibong et al., 1986).

Ova of pubertal gilts are fertilized at approximately the same rate as those of older gilts (Archibong et al., 1987). However, fertilization followed by normal cleavage does not preclude the possibility that ova of pubertal gilts may carry some induced defect that is expressed at a later stage of embryonic development. Andersson and Einarsson (1980) reported that peripubertal gilts have a longer proestrus than those that have cycled beyond second estrus. This observation is consistent with the finding that serum concentrations of estradiol in peripubertal gilts peak 2.5 d before puberty (Esbenshade et al., 1982), whereas in cyclic gilts peak levels of this steroid occur 24 h before onset of estrus (Henricks et al., 1972). Consequently, oocytes of pubertal gilts may become defective due to prolonged exposure to steroids and(or) other agents produced by the maturing follicle. Inducing early ovulation in the pubertal gilt by administration of appropriate exogenous hormone on the first day of detected estrus might therefore reduce the incidence of embryonic mortality in this animal. Thus, the objective of the present study was to evaluate the effect of gonadotropin releasing hormone (GnRH) on ovulation and embryonic survival in pubertal gilts.
Materials and Methods

Twenty prepubertal gilts (73 to 75 kg body weight) were fed ad libitum a balanced commercial diet containing 15% protein until attainment of puberty. Subsequently they were fed a restricted diet of 2.5 kg·head⁻¹·d⁻¹ containing 16% protein.

Prepubertal gilts were housed adjacent to a boar and checked twice daily for estrus by application of hand pressure on each gilt's back in the presence of a boar. Gilts were mated to boars of established fertility initially at the time of detected estrus (d 0 of gestation) and subsequently at 12 and 24 h after detected estrus. After initial mating, gilts were assigned in replicates to a treatment (n=10) or control (n=10) group, weighed, and the treated gilts were injected via an ear vein with 200 μg of GnRH; control gilts were similarly injected with physiological saline (vehicle). Blood samples (approximately 25 ml) were collected by the method of Schwartz and Smallwood (1977) from the anterior vena cava of five treated and five control gilts (chosen randomly) immediately prior to injection (0 min), thereafter at 15-min intervals for 90 min, and subsequently from all gilts just before slaughter on d 30 of gestation. Blood samples were held at room temperature (25 C) for 12 h, then at 4 C for 48 h, after which time they were centrifuged at 500 × g for 10 min at 4 C. The resulting sera were stored at -20 C until assayed for luteinizing hormone (LH) and progesterone.

Ovaries and uteri were removed from gilts 15 to 20 min after slaughter and placed on crushed ice until processed. Ovulation rate of each gilt was determined by counting the number of corpora lutea in each ovary. Pubertal status was confirmed by the absence of corpora albicantia in the ovaries of both control and treated gilts at slaughter. Corpora lutea from both ovaries of each gilt were then enucleated and weighed. Conceptuses were exposed by an incision made along the length of each uterine horn and examined for viability. Fetuses were considered to be viable if they exhibited characteristics described by Marrable (1971). Number of viable fetuses was recorded and percentage viable fetuses on d 30 of gestation was determined as follows: (number of viable fetuses/number of corpora lutea) × 100.

Radioimmunoassay. Sera collected from gilts before and after injection of GnRH or vehicle on the day of detected estrus were analyzed for LH only; sera collected from all treated and control gilts on d 30 of gestation were analyzed for progesterone. Sera were analyzed for LH and progesterone using radioimmunoassays described by McCarthy and Swanson (1976) and Koligian and Stormshak (1977), respectively.

Recovery (~ ± SE) of various quantities of LH (0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1, 2, 4, 8 and 10 ng) added to 200 μl of pooled serum obtained from sows at the midluteal phase of the estrous cycle were: 0.13 ± 0.02, 0.23 ± 0.02, 0.30 ± 0.03, 0.39 ± 0.01, 0.55 ± 0.03, 0.74 ± 0.04, 0.98 ± 0.04, 1.96 ± 0.03, 3.93 ± 0.05, 7.30 ± 0.20 and 11.08 ± 0.64 ng, respectively. The antibody (no. 566, prepared against a porcine LH fraction) utilized was previously shown to be highly specific for porcine LH (Niswender et al., 1970). Sensitivity of this assay was 0.1 ng/tube (P<.01, n = 10). The intra-assay coefficient of variation for the LH assay was 2.5%. There was no inter-assay coefficient of variation for LH because all samples were analyzed in the same assay. The assay for progesterone was previously validated for porcine sera by Archibong et al. (1987). Intra- and inter-assay coefficients of variation for the progesterone assay were 6.8 and 15.8%, respectively.

Statistical Analysis. Data on serum concentrations of LH were analyzed by split-plot analysis of variance. Data on serum concentrations of LH before GnRH and vehicle injection, ovulation rate (number of corpora lutea), number of attached conceptuses, percentage of viable fetuses, weight of corpora lutea and serum concentrations of progesterone were analyzed by use of Student's t-test.

Results and Discussion

Gilts treated with GnRH weighed 98.9 ± 2.0 kg (X ± SE); control gilts weighed 91.0 ± 3.6 kg at puberty. These weights did not differ statistically and were comparable to those of pubertal gilts at this station reported by Knott et al. (1984).

Serum concentrations of LH prior to injections in both GnRH-treated and control gilts (7.2 ± 1.5 vs 4.3 ± 2.1 ng/ml; figure 1) did not differ (P>.05) and were similar to peak concentrations of this hormone at the onset of estrus in gilts as reported by Niswender et al. (1970). These initial serum concentrations of LH
suggest that gilts in the present experiment may have already been releasing near-maximal quantities of LH at the time of the injections. Treatment with GnRH increased secretion of LH during the 90 min after injection (time x treatment interaction, $P<.025$). The higher serum concentrations of LH after GnRH treatment are in agreement with the data of Chakraborty et al. (1973), Andersson et al. (1983) and Lutz et al. (1985), with the highest serum concentrations detected at 75 min after GnRH injection. In control gilts, serum concentrations of LH after injection of saline were similar to those detected prior to injection. These data are in agreement with those of Niswender et al. (1970), who determined that serum concentrations of LH remained elevated for 8 h after peak concentrations at the onset of estrus.

Gonadotropin releasing hormone-induced increase in secretion of LH was accompanied by a concomitant increase ($P<.05$) in ovulation rate (table 1). These data confirm, in part, those of Edquist et al. (1978) and Lutz et al. (1985), who reported that exogenous GnRH caused ovulation in prepubertal gilts. The increase in ovulation rate in GnRH-treated gilts in the present study resulted from the ovulation of ovarian follicles that otherwise would not have ovulated. This suggests that in the normal prepubertal gilt the population of follicles capable of being ovulated is greater than the actual number of ovulations that occur in response to the quantity of gonadotropin released.

Mean number of attached conceptuses recovered on d 30 of gestation, as well as the percentage of viable fetuses, did not differ between GnRH-treated and control gilts (table 1). Number of surviving embryos in pubertal gilts was expected to increase with increased number of ovulations, as recently reported by Archibong et al. (1987). In this latter study, embryonic survival in third-estrous gilts, which had similar numbers of ovulations as compared with those gilts treated with GnRH in the present study, was greater than that of gilts mated at puberty. Because embryonic survival did not accompany increased ovulation rate in GnRH-treated gilts, it is possible that ova in these gilts may have already been defective before GnRH treatment. Alternatively, ovulation of extra follicles due to GnRH treatment may have resulted in asynchrony of development among embryos. The few additional ova ovulated as a result of GnRH treatment may have been fertilized later than normal. Consequently, there may have existed a mixed population of embryos, differing sufficiently in age such that the uterine environment favored survival of embryos resulting from early ovulation and fertilization. Pope et al. (1982) demonstrated that older embryos create a uterine

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>GnRH</th>
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</thead>
<tbody>
<tr>
<td>Ovulation rate</td>
<td>12.1 ± .6</td>
<td>14.5 ± .7*</td>
</tr>
<tr>
<td>No. of attached conceptuses</td>
<td>10.9 ± .7</td>
<td>10.9 ± .9</td>
</tr>
<tr>
<td>Viable fetuses, %</td>
<td>83.5 ± 5.0</td>
<td>74.7 ± 6.9</td>
</tr>
<tr>
<td>Corpora lutea wt, mg</td>
<td>389.3 ± 11.3</td>
<td>402.8 ± 16.3</td>
</tr>
<tr>
<td>Serum progesterone, ng/ml</td>
<td>22.1 ± 2.9</td>
<td>25.0 ± 2.6</td>
</tr>
</tbody>
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* $P<.05$. 

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*Figure 1. Changes in serum concentrations of LH (mean ± SE) in control and GnRH-treated gilts on the first day of pubertal estrus. Vehicle (saline) or GnRH (200 µg) were injected iv at time 0.*
environment that is not conducive to development of younger embryos. Asynchronous cohabitation of embryos at diverse stages of development, coupled with other agents that normally impact on embryonic survival, may have contributed to the demise of more embryos among the GnRH-treated gilts.

Control and treated gilts did not differ in mean weight of corpora lutea (table 1). Similarly, mean serum levels of progesterone (ng/ml; table 1) did not differ between GnRH-treated and control gilts. These data are in agreement with those of Archibong et al. (1987), who found that an increase in number of corpora lutea was not accompanied by a significant increase in serum concentrations of progesterone on d 30 of gestation. Data from the present study indicate that uteri of control and treated gilts should have been exposed to similar systemic concentrations of progesterone. Whether progesterone-stimulated production of some uterine factor essential for embryonic survival (Bazer et al., 1982; Bazer and Roberts, 1983) was limiting relative to the greater embryo population resulting from higher ovulation in GnRH-treated gilts is not known.

Results of this study indicate that administration of GnRH to gilts mated at the onset of pubertal estrus induced increased secretion of LH and number of ovulations. However, the increased ovulation rate was not accompanied by increased embryonic survival or litter size.

**Literature Cited**


