OVARIAN RESPONSE TO PREGNANT MARE SERUM GONADOTROPIN AND PORCINE PITUITARY EXTRACT IN GILTS ACTIVELY IMMUNIZED AGAINST GONADOTROPIN RELEASING HORMONE\textsuperscript{1,2}

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ABSTRACT

Two experiments were conducted to determine the effect of exogenous gonadotropins on follicular development in gilts actively immunized against gonadotropin releasing hormone (GnRH). Four gilts, which had become acyclic after immunization against GnRH, and four control gilts were given 1,000 IU pregnant mare serum gonadotropin (PMSG), while four additional control gilts were given saline. Control animals were prepuberal crossbred gilts averaging 100 kg body weight. Control gilts given saline had ovaries containing antral follicles (4 to 6 mm in diameter). Control gilts given PMSG exhibited estrus and their ovaries contained corpora hemorrhagica and corpora lutea. PMSG failed to stimulate follicular growth in gilts immunized against GnRH, and ovaries contained regressed corpora albicantia and small antral follicles (<1 mm in diameter). Concentrations of luteinizing hormone (LH) and estradiol-17\beta (E\textsubscript{2}) were non-detectable in gilts immunized against GnRH and given PMSG. In the second experiment, five gilts actively immunized against GnRH were given increasing doses of PMSG every third day until unilateral ovariectomy on d 50. PMSG failed to stimulate follicular growth, and concentrations of follicle stimulating hormone (FSH), E\textsubscript{2} and LH were not detectable. Six weeks later, gilts were given a booster immunization and then were given 112 \mu g LH and 15 \mu g FSH intravenously every 6 h for 9 d. The remaining ovary was removed on d 10. Although LH and FSH concentrations were elevated, administration of gonadotropins did not stimulate follicular growth or increase E\textsubscript{2} concentrations. These results indicate that neither PMSG or exogenous LH and FSH can induce E\textsubscript{2} synthesis or sustain follicular development in gilts actively immunized against GnRH.

(Key Words: Pigs, Gonadotropin Releasing Hormone, PMSG, Follicles.)

Introduction

Pregnant mare serum gonadotropin (PMSG) has been used to induce follicular growth in prepuberal (Dziuk and Gehlbach, 1966), puberal (Baker and Coggins, 1968) and anestrous (Dziuk and Dhindsa, 1969) gilts and in anestrous lactating (Hausler et al., 1980; Hodson et al., 1981) and weaned (Dial et al., 1984) sows. Follicular growth also has been stimulated in prepuberal gilts by giving porcine pituitary extract (Kraeling, 1970), and frequent pulses of gonadotropin releasing hormone (GnRH; Carpenter and Anderson, 1985; Lutz et al., 1985), in postpartum sows by a crude follicle stimulating hormone (FSH) preparation (Peters et al., 1968, 1969), and in anestrous lactating and weaned sows by pulsatile administration of GnRH (Cox and Britt, 1982; Armstrong and Britt, 1985).

In contrast, PMSG did not stimulate follicular growth in gilts after hypophysectomy (Kraeling et al., 1974) or hypophyseal stalk transection (Kraeling et al., 1986). The objective of the present study was to determine if the pig responds to PMSG and exogenous pituitary extracts after active immunization against GnRH.
Materials and Methods

Exp. 1. Four cyclic crossbred gilts, approximately 11 mo of age, were actively immunized against GnRH according to the procedures of Esbenshade and Britt (1985). These animals were acyclic and had non-detectable concentrations of gonadotropins at the beginning of the experiment. Eight prepuberal crossbred gilts averaging 100 kg body weight were used as controls. Four gilts actively immunized against GnRH and four control gilts were each given 1,000 IU PMSG im on d 0. The other four control gilts were given saline. All gilts were observed for estrus once daily in the presence of a boar. Ovaries were removed from gilts immunized against GnRH by ovariectomy and from control gilts by slaughter on d 5. Gilts immunized against GnRH were non-surgically cannulated before beginning the experiment and blood samples were collected every 8 h from these gilts.

Exp. 2. Five crossbred gilts, actively immunized against GnRH approximately 6 mo previously, were given a booster immunization 7 d before initiation of this experiment. Gilts were given PMSG im every third day as follows: 200 IU on d 0, 3, 6, 9 and 12; 400 IU on d 15, 18, 21, 24 and 27; 800 IU on d 30, 33, 36, 39 and 42; and 1,600 IU on d 45 and 48. Blood samples were taken by venipuncture on d 8, 37 and 42 and all gilts were unilaterally ovariectomized on d 50. Gilts were observed for estrus once daily in the presence of a boar.

Approximately 6 wk after the gilts were unilaterally ovariectomized, they were given a booster immunization on d -4, where d 0 was the day of non-surgical cannulation of the vena cava. Blood samples were taken every 6 h beginning at 1800 of d 0, which was approximately 9 h after cannulation, and continuing until 0600 of d 10. Two milliliters of a suspension of pituitary extract was given iv every 6 h, immediately after taking each blood sample, from 0600 on d 1 to 1800 on d 9. The remaining ovary was removed on d 10.

Pituitary Extract. Lyophilized porcine pituitaries were donated. The pituitary powder was added to saline at a rate of 5.4 g in 430 ml. The mixture was stirred for 2 h at room temperature and centrifuged at 2,000 x g for 30 min. The supernatant was decanted, passed through a .45-μm millipore filter and divided into 10 ml aliquots. Two milliliters of the suspension was given at each infusion time. The 2-ml aliquot contained 112 μg LH and 15 μg FSH as determined by the following radioimmunoassay procedures.

Blood Samples and Radioimmunoassays. Blood samples were allowed to clot at 4 C and serum was collected by decanting the supernatant following centrifugation. Serum was stored at −20 C until assayed.

Serum LH was quantified by radioimmunoassay (Niswender et al., 1970; Stevenson et al., 1981), with porcine LH (LER-786-3) used as the radioiodinated antigen and standard. Average sensitivity of the assays was .3 ng/ml.

Serum concentrations of FSH were determined by radioimmunoassay procedures using anti pFSH (USDA-10-1010) and 125I-pFSH (USDA-FSH-PP1; Guthrie and Bolt, 1983) as modified by Esbenshade and Britt (1985). Average sensitivity of the assay was .5 ng/ml. All samples were analyzed in one assay and intra-assay coefficients of variation calculated from seven replicates were 11 and 10% for LH and FSH, respectively.

A previously validated radioimmunoassay was used to quantify serum concentrations of estradiol-17β (Cox and Britt, 1982).

Preparation of Ovaries. Ovaries were trimmed of excess tissue, fixed in Bouins for 4 to 12 h, washed several times in 50% ethanol, and stored in 70% ethanol until processed. Ovaries from gilts in Exp. 2 were weighed before fixation. A mid-sagittal section, 10 μm thick, was taken from each ovary in Exp. 1, while ovaries from gilts in Exp. 2 were sectioned serially at 10 μm. Every 10th section was mounted and stained with hematoxylin for histological examination. All follicles with more than four layers of granulosa cells were counted, grouped as antral, beginning antral or nonantral follicles and classified as either atretic or nonatretic. A follicle was classified atretic when more than four pyknotic bodies were observed in the section that contained the oocyte.

Statistical Analysis. Differences in ovarian weight and classification of follicles after treatment with PMSG or pituitary extract were compared by Student’s t-test.

Results

Exp. 1. All control gilts given PMSG exhibited estrus 4 d after treatment. In contrast,
Figure 1. Photomicrographs (approximately 10×) of ovaries from a control gilt given saline (A), control gilt treated with PMSG (B) and GnRH-immunized gilt treated with PMSG (C).
none of the control gilts given saline and none of the gilts actively immunized against GnRH showed estrus.

Photomicrographs of ovaries from the three treatment groups are presented in figure 1. Ovaries from control gilts contained numerous 4- to 6-mm antral follicles. PMSG induced follicular development and ovulation in control gilts with 7.5 ± 1.0 (X ± SE) corpora hemorrhagica and(or) corpora lutea observed on the ovaries of each gilt. Ovaries from gilts immunized against GnRH and given PMSG had no morphologically distinct structures on their surfaces. Histological observation revealed that these ovaries contained regressed corpora albicantia and small antral follicles less than 1 mm in diameter.

Serum concentrations of LH and estradiol-17β taken at 8-h intervals from gilts immunized against GnRH and given PMSG were below the sensitivity of the assays and were recorded as non-detectable.

Exp. 2. None of the gilts exhibited estrus during treatment with PMSG or pituitary extract. Table 1 summarizes serum concentrations of LH, FSH and estradiol-17β during treatment with PMSG and ovarian weights after unilateral ovariectomy. Concentrations of LH increased during the experimental period, while FSH and estradiol-17β were not detectable. Ovaries were taken from gilts after PMSG treatment had no morphologically distinct surface structures.

Figure 2 illustrates inhibition curves for pLH and PMSG. PMSG reacted with the porcine anti-LH serum to yield an inhibition curve between .035 and 14 IU/tube for 90 and 20% binding, respectively.

Mean serum concentrations of LH and FSH during administration of pituitary extract were 6.3 (range 2.4 to 16.4) and .6 (range .3 to 2.0) ng/ml, respectively (figure 3). Estradiol-17β concentrations were below the sensitivity of the assay for all samples.

The remaining ovary taken from each of the gilts had no morphologically distinct surface structures. Mean (± SE) weight of the remaining ovaries taken from gilts after treatment with LH and FSH was 1.6 ± .2 g; these ovaries were heavier than ovaries taken from the same gilts after PMSG administration (.9 ± .1; P<.02).

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**TABLE 1. CONCENTRATION OF HORMONES AND OVARIAN WEIGHT OF GILTS ACTIVELY IMMUNIZED AGAINST GnRH AND GIVEN MULTIPLE DOSES OF PMSG**

<table>
<thead>
<tr>
<th>Item</th>
<th>8</th>
<th>37</th>
<th>42</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH, ng/ml</td>
<td>ND</td>
<td>2.42 ± .65c</td>
<td>3.64 ± .86c</td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Estradiol-17β</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ovarian wt, g</td>
<td></td>
<td></td>
<td></td>
<td>.9 ± .1d</td>
</tr>
</tbody>
</table>

*First day of treatment with PMSG = d 0.

bND = non-detectable.
cMean ± SE; N=5.
dMean ± SE; N=4.
Figure 3. Mean serum concentrations of LH (---) and FSH (---) in gilts given pituitary extract. Standard errors were proportional to the mean, and ranged from .1 to 4.8 and .07 to 1.39 for LH and FSH, respectively.

Ovaries taken from gilts treated with pituitary extract had significantly more nonantral follicles, but there was no difference in the number of beginning antral or antral follicles for the two treatment groups (table 2). Most follicles from all stages of development were classified as atretic and there was no difference between treatment groups in the percentage of atretic follicles.

Discussion

Administration of PMSG, either as a single intramuscular injection or as multiple injections over a period of 50 d, failed to induce follicular development in gilts actively immunized against GnRH. Ovaries from GnRH-immunized gilts given PMSG were smaller in size and weight than ovaries from gilts of comparable age and weight (Dyck and Swierstra, 1983). There were no morphologically distinct surface structures on the ovaries and histological examination revealed many small primary and secondary follicles dispersed throughout the interstitium, with several antral follicles less than 1 mm in diameter. A similar lack of response to PMSG has been reported in the hypophysectomized gilt (Kraeling et al., 1974). In that study, a single injection of PMSG 2 or 5 d after hypophysectomy failed to stimulate follicular growth. These results suggest that the ovary cannot undergo follicular growth following acute or chronic deprivation of gonadotropins. Presumably, there was irreversible loss of gonadotropin receptors on the ovary, although treatments used in these studies may not have been of sufficient intensity or duration to overcome the complete loss of gonadotropin support by immunoneutralization of GnRH or hypophysectomy.

Comparison of ovarian weights of experimental animals after multiple PMSG injections and after intravenous administration of exogenous gonadotropin revealed that the second ovary removed was significantly heavier than the first. A direct count of all follicles with more than four layers of granulosa cells revealed that treatment with pituitary extract induced more follicles from the pool of primary follicles than did treatment with PMSG. Because there was no difference between treatment groups in the number of beginning antral or antral follicles, this suggests an acute effect of exogenous gonadotropin treatment on the early stages of follicular development. If exogenous LH and FSH stimulated division of granulosa cells and early follicular growth, the gonadotropins were not effective in maintaining

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Nonantral</th>
<th>Beginning antral</th>
<th>Antral</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG</td>
<td>4</td>
<td>87 ± 16e</td>
<td>59 ± 18 (96)</td>
<td>63 ± 17 (97)</td>
</tr>
<tr>
<td>Pituitary extract</td>
<td>5</td>
<td>135 ± 13d</td>
<td>56 ± 14 (97)</td>
<td>62 ± 11 (98)</td>
</tr>
</tbody>
</table>

aValues represent mean ± SE.
bValues in parentheses represent percentage of follicles classified as atretic.
cdValues with different superscript differ (P<.02).
viability of these follicles, as evidenced by the same degree of atresia in pre-antral follicles as in beginning antral and antral follicles.

Because concentrations of gonadotropins and gonadal steroids were eliminated by active immunization against GnRH, ovarian compensatory hypertrophy could have been caused by factors other than gonadotropin replacement therapy. One such possibility is sensory innervation of the ovary. In the rat, the vagus nerve relays sensory signals from the ovary (Burden et al., 1983), because vagotomy alters estrous cyclicity (Burden et al., 1981), blocks induction of pseudopregnancy (Burden et al., 1981), delays onset of puberty (Ojeda et al., 1983), increases fetal resorption during pregnancy (Lawrence et al., 1978) and prevents compensatory ovarian hypertrophy (Burden and Lawrence, 1977). If a similar mechanism exists in the pig, the increase in weight of the second ovary following removal of the first in the present study may have been mediated through a neuronal mechanism.

Because PMSG cross-reacted with the LH antibody, it was possible to detect increasing concentrations of exogenous gonadotropin during repeated injections of this compound. This observation, along with the measurable concentrations of LH and FSH in the second part of Exp. 2, suggest that gonadotropins alone are not sufficient to sustain follicular development beyond 1 mm in diameter nor to prevent follicles from becoming atretic. In addition, these treatments were not sufficient to induce steroidogenesis, as evidenced by the lack of estrogen production.

**Literature Cited**


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