REGRESSION OF CORPORA LUTEA IN PREGNANT GILTS ADMINISTERED ANTIOVINE LH RABBIT SERUM

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LUTEOTROPIC properties of luteinizing hormone (LH) based on elevated steroidogenesis of luteal tissue in vitro have been reported in a number of species (Armstrong, 1966; Marsh and Savard, 1966). Du Mesnil du Buisson et al. (1964) have reported that LH preparations maintained corpora lutea to the 20th day in hypophysectomized-hysterectomized pigs; however, they could not maintain them beyond this stage.

The changing levels of LH and luteotropin at the time of ovulation appear similar in the pig (Brinkley et al., 1964; du Mesnil du Buisson and Léglise, 1963). Sammelwitz et al. (1961) have hypothesized that the release of hypophysial luteotropin occurs as a single surge near the time of ovulation followed by a subsequent release, only if the gilt becomes pregnant or is hysterectomized (du Mesnil du Buisson, 1964). The second phase of luteotropic release is presumed to be continuous, since hypophysectomy (du Mesnil du Buisson and Léglise, 1963) or the administration of exogenous progesterone (Spies et al., 1959, 1960; Sammelwitz et al., 1961) at this stage results in regression of corpora lutea. Evidence suggests the exogenous progesterone acts by blocking pituitary LH (Foote et al., 1958; Parlow et al., 1964; Anderson et al., 1966). Differences in concentrations of pituitary hormones, without comparable information on plasma level hormones, need to be interpreted conservatively. However, Melampy et al. (1966) reported that pituitary LH concentration remains high between day 18 to 25 of pregnancy, but decreases between day 18 of one estrous cycle and day 1 of the next estrous cycle (Parlow et al., 1964). These data indicate that LH may have luteotropic properties in the pig, but more conclusive evidence is needed to establish it as the only hormone needed for luteal maintenance and function.

Antagonadotropin has been reported to block ovulation and inhibit estrus in rats (Bourdel and Li, 1963; Kelly et al., 1963) and to block ovulation in rabbits (Quadri et al., 1966). Also, Rennels (1964) reported that anti-LH inhibited ovarian ascorbic acid depletion in LH-treated immature rats. Although these preparations were not specific for one antibody, the major effects were suggestive of LH inhibition.

The present experiments were designed to test the effect of ovine luteinizing hormone (LH) antiserum on ovulation and in estrous gilts, and luteal function in pregnant and open gilts, and to compare luteal regression evoked by progesterone with that induced by anti-LH.

Materials and Methods

Antiserum was prepared in two female rabbits by four weekly subcutaneous injections of 1.5 mg. LH 2 dissolved in 3.0 ml. of a 1:1 mixture of saline and Freund's complete adjuvant. Two additional booster injections of 2 mg. each were given 1 week apart at 13 and 14 weeks. Twenty-five to 50 ml. of blood were collected from each rabbit 5 days following each booster injection. The globulin fraction was separated from the antiserum and concentrated to one-third saturation by repeated ammonium sulfate precipitation, using the procedure described by Campbell et al. (1964). The final precipitate was dissolved in borate-buffered saline at one-third the original serum volume. The sulfate ions were removed by dialysis at 4ºC., and aliquots of the preparation were pooled and stored at 4ºC. until used. Total protein was not determined nor was any attempt made to purify the antiserum. A gel diffusion test as described by Ouchterlony (1953) was conducted with the antiserum against ovine LH, FSH, TSH, STH and prolactin. An interfacial test (Campbell et al., 1966) was used to test the specificity of the antiserum.

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2 Luteinizing hormone preparation used was NIH-LH-S4.

3 NIH-LH-S10 was a gift of the Endocrinology Study Section of National Institutes of Health.

4 Difco, Detroit, Michigan.

5 Ovine follicle stimulating hormone (NIH-FSH-S1), thyroid stimulating hormone (NIH-TSH-S1), somatotropin (NIH-2170-C1-3) and prolactin (NIH-P-S-7) were graciously provided by Dr. Leo Reichert, Jr., Emory University.

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Adjuvant rabbit serum was prepared in the same manner as described for the antiserum, except only Freund's complete adjuvant was used.

Poland China and Poland China-Duroc crossbred gilts were used. Estrus was detected by observing the gilts' behavior once daily with the aid of an intact boar. Coitus was allowed only when a gilt was to be assigned to one of the pregnant groups. The first day of estrus was considered day 1.

Four unmated gilts were injected with 1 ml. of the antiserum preparation (equivalent to 3 ml. of original undiluted serum) intravenously immediately upon detection of estrus, in an attempt to block ovulation. The gilts were slaughtered 3 days after estrus and their ovaries examined for recently formed corporea lutea.

Six unmated gilts were divided into two equal groups. One group was untreated and the other group received 1 ml. of the antiserum preparation intravenously daily on days 7 through 11 of the estrous cycle. Both groups were slaughtered on day 12. The corpora lutea were dissected from the surrounding ovarian tissue, counted, weighed and stored in ethanol at 5°C until progesterone assay (Zander, 1962). Tritium-labeled progesterone was added to each sample prior to initial extraction, and the final progesterone concentrations reported were adjusted for recovery rates.

Twenty gilts were bred to a fertile boar. The three gilts which returned to estrus by the 24th day after mating were eliminated: the remaining 17 gilts were assigned at random to one of the following five treatment groups: (1) untreated, (2) 1 ml. adjuvant serum injected intravenously, (3) 1 ml. of antiserum intravenously, (4) 1 ml. antiserum plus 400 mg. progesterone injected subcutaneously and (5) 400 mg. progesterone alone. Daily treatment was begun on the 25th day after mating and continued through day 29. The animals were slaughtered on day 30. Corpora lutea were handled as described above. Embryos and membranes were removed from the uteri, counted and weighed.

Mean weights and concentrations of progesterone of corpora lutea were analyzed for differences in treatment by analysis of variance. Mean concentration of progesterone was transformed to logarithms for the analysis, since the larger means had greater standard errors. The data for unmated and pregnant gilts were analyzed separately. Survival rate of embryos for gilts in the pregnant groups was tested for significance by analysis of variance after transforming the percentages to arc sin to provide a more normal distribution. Embryo and membrane weights of only the 12 animals pregnant at slaughter were included in the statistical test for group differences. A multiple range test was used to compare group means where analysis of variance indicated a significant difference.

Results

Ovulation was inhibited in two of four gilts injected with 1 ml. of antiserum on the first day of estrus. New corpora lutea were present in the remaining two gilts, and ova were recovered from the oviducts.

Average weights of corpora lutea and concentrations of progesterone were, respectively, 383 mg. and 47.7 mcg. for the untreated, unmated group, and 426 mg. and 28.1 mcg. for the antiserum-treated unmated gilts (table 1). These means did not differ significantly (P>.05).

Average weights and concentrations of progesterone of corpora lutea, percentages of embryo survival, and embryo and membrane weights for the five groups of pregnant gilts are shown in table 1. Average weights of corpora lutea of groups treated with progesterone and progesterone plus antiserum differed significantly (P<.05) from the average luteal weights of the untreated and adjuvant serum-treated groups. Antiserum alone did not decrease luteal weight significantly (P=.07) below that in the untreated group. Progesterone concentrations were significantly reduced by antiserum, antiserum plus progesterone and progesterone alone (table 1). The luteal tissue in one pregnant animal in the adjuvant serum-treated group had an unusually high progesterone content. This value was included in the calculation of the group mean, thus accounting for the high mean value.

No embryos survived in the antiserum-treated group. One of three gilts administered adjuvant serum was not pregnant when slaughtered. The weight of her corpora lutea and the concentration of progesterone suggested that luteal regression had not occurred. Neither the four antiserum-treated gilts nor the non-pregnant adjuvant serum-treated gilt were in estrus prior to slaughter.

Exogenous progesterone maintained preg-
TABLE 1. AVERAGE WEIGHTS OF CORPORA LUTEA, PROGESTERONE CONCENTRATIONS, EMBRYO SURVIVAL AND EMBRYO AND MEMBRANE WEIGHTS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Corpora lutea</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no.</td>
<td>Gilts</td>
<td>Mean wt.</td>
<td>Prog. conc.</td>
<td>Survival</td>
<td>Mean wt.</td>
</tr>
<tr>
<td>Unmated gilts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>3</td>
<td>383</td>
<td>47.7±6.5*</td>
<td></td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>Antiserum*</td>
<td>3</td>
<td>426</td>
<td>28.1±3.0</td>
<td></td>
<td>....</td>
<td>....</td>
</tr>
<tr>
<td>Pregnant gilts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>3</td>
<td>370</td>
<td>44.7±11.9*</td>
<td></td>
<td>64.6</td>
<td>1.367</td>
</tr>
<tr>
<td>Adjuvant serum*</td>
<td>3</td>
<td>388</td>
<td>64.4±12.1*</td>
<td></td>
<td>63.7</td>
<td>0.827</td>
</tr>
<tr>
<td>Antiserum+progesterone</td>
<td>4</td>
<td>226</td>
<td>8.2±3.3</td>
<td></td>
<td>0</td>
<td>....</td>
</tr>
<tr>
<td>Progesterone</td>
<td>3</td>
<td>135</td>
<td>6.6±1.5</td>
<td></td>
<td>75.3</td>
<td>1.083</td>
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<tr>
<td></td>
<td>3</td>
<td>151</td>
<td>10.1±4.8</td>
<td></td>
<td>63.7</td>
<td>0.645</td>
</tr>
</tbody>
</table>

*a, b Means of the same column without a common superscript differ significantly (P<.05).
*1 ml. once daily intravenously on days 7 through 11 of the estrous cycle.
*1 ml of adjuvant serum or antiserum once daily intravenously on days 25 through 29; progesterone, when given, was subcutaneous once daily at 400 mg. during the same time interval.
*Standard error of the mean.
*Percent embryo survival is expressed as the number of live embryos at slaughter divided by the number of corpora lutea X 100.

Discussion

Reduced corpora lutea weight and progesterone concentration accompanied by complete loss of embryos indicate antiserum against ovine LH was capable of inhibiting luteotropic function in pregnant gilts. It is unlikely that any direct toxic effect of rabbit serum or Freund's adjuvant on the embryo is responsible for these effects, since progesterone maintained pregnancy in the presence of antiserum. Furthermore, neither mean embryo survival rate nor luteal progesterone concentration was suppressed in adjuvant serum-treated gilts. Also, the weights of embryos and membranes were not significantly affected by adjuvant serum or antiserum plus progesterone when compared to weights of the control group. The reduced progesterone concentration and corpora lutea weights following treatment with exogenous progesterone in the pregnant gilts agree with previous reports (Spies et al., 1959; Sammelwitz et al., 1961). The data show no indication of synergistic inhibitory action between antiserum and progesterone, and are compatible with the hypothesis that either treatment effectively blocks luteal function in the pregnant pig, although the sites of their blocking action may differ.

Failure of the antiserum to significantly influence the weight of corpora lutea or progesterone concentration in unmated gilts supports the hypothesis that corpora lutea of the cycle do not require LH, nor supposedly luteotropin, subsequent to ovulation (Sammelwitz et al., 1961). The antiserum blocked ovulation in only two of four gilts, perhaps because the ovulating surge of LH occurs near onset of estrus in the pig (du Mesnil du Buisson and Léglise, 1963), and the antiserum may have been given subsequent to the action of LH. Quadri et al. (1966) reported inhibition of ovulation in rabbits when aliquots of antiserum, prepared in a manner similar to that employed in the current study, were injected intravenously at the time of mating.

These data are not conclusive evidence that LH is the only luteotropic hormone in the pig, since a gel diffusion test of the antiserum with various NIH ovine pituitary preparations revealed precipitin bands against ovine FSH, TSH and possibly STH, in addition to LH, but not against ovine prolactin. Thus, it is unlikely that the luteal regression prompted by the antiserum is related to inhibition of endogenous prolactin.

Summary

Treatment with antibodies to ovine LH resulted in atrophy of corpora lutea and complete loss of embryos in pregnant gilts. Exogenous progesterone prevented the loss of embryos caused by the antiserum. One millilitre antiserum daily had no significant effect on weight of corpora lutea or concentration of
progesterone in gilts treated during the luteal phase of the estrous cycle, but did block ovulation in two of four gilts when injected intravenously near the onset of estrus. The data suggest that ovine LH antiserum inhibits the hormone(s) required for luteal function in the pregnant gilt. Since the antiserum was not purified, the data do not define the specific nature of luteotropin in the pig, but suggest that LH is one hormone involved in luteal function.

Literature Cited


