SERUM LH IN EWES TREATED WITH SYNTHETIC LUTEINIZING HORMONE-RELEASING HORMONE/FOLLICLE STIMULATING HORMONE-RELEASING HORMONE (LH-RH/FSH-RH) AT THREE PERIODS OF ANESTRUS

Jerry J. Reeves, Gladys K. Tarnavsky and Prabir K. Chakraborty
Washington State University, Pullman 99163

Summary

Ten anestrous ewes were assigned at random to two equal groups. Treatments consisted of a single intramuscular injection of either 50μg synthetic luteinizing hormone-releasing hormone/follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH) in acidified saline or an equivalent volume of acidified saline. Blood samples were collected via jugular puncture immediately preceding and at 30 min intervals after treatment for a subsequent 8 hours. The previously described treatments were repeated three times at 45-day intervals and were designated as early (May 7), mid (June 21) and late (August 5) anestrus. Each ewe received the same treatment for all three stages of anestrus. All ewes were laparotomized before and after treatment to determine ovulation rate. Each serum sample was assayed for LH by a radioimmunoassay and the pretreatment samples for the LH-RH/FSH-RH group were quantitated for total estrogens by a radioimmunoassay. The mean heights of the peak LH found after LH-RH/FSH-RH treatment in early, mid and late anestrus were 70.8 ± 16.7, 64.8 ± 13.2 and 93.6 ± 20.0 ng/ml, respectively, and were significantly higher than control values. Pituitary responsiveness to LH-RH/FSH-RH was not different at the three stages of anestrus as indicated by lack of significant differences among mean peak serum LH concentrations. Mean serum total estrogen concentrations before treatment with LH-RH/FSH-RH were not significantly altered due to stage of anestrus. Endogenous estrogen levels before treatment did not appear to affect the response to LH-RH/FSH-RH as suggested by a correlation coefficient of r = -.243 between estrogen levels before treatment and peak serum LH response to LH-RH/FSH-RH. The circulating levels of estrogens in the anestrous ewes were very low and were measured at the lower limit of assay sensitivity. Thus lack of significant correlation between pretreatment endogenous estrogen levels and response to LH-RH/FSH-RH may not indicate lack of estrogen effect. One ewe out of five ovulated after LH-RH/FSH-RH treatment at each stage of anestrus compared to no ovulation in the saline treated ewes. None of the ewes including those which ovulated exhibited estrus after LH-RH/FSH-RH treatment.

Introduction

Domanski and Kochman (1968) found that when a hypothalamic extract was infused into the pituitary of ewes during mid anestrus no ovulation was induced but the same extract did induce ovulation toward the end of the anestrous season. They interpreted these data as indicating that a change in the pituitary responsiveness to the hypothalamic LH releasing hormone had occurred during the anestrous season. Reeves, Arimura and Schally (1971a) using cycling ewes, also found changes in pituitary responsiveness to purified porcine LH-RH during the estrous cycle of the ewe. Later results suggested that this change in pituitary responsiveness to LH-RH could be influenced by pretreatment of the ewes with estrogen (Reeves et al. 1971b).

The present study was designed to evaluate changes in pituitary responsiveness in ewes to...
synthetic LH-RH/FSH-RH at three different times during the anestrous season and to determine if endogenous serum estrogen concentrations were correlated with pituitary responsiveness to LH-RH/FSH-RH.

Materials and Methods

Experimental Animals Ten 16-month-old Columbia ewes averaging 55 kg body weight were assigned at random to two treatment groups. The ewes were kept throughout the experiment with a vasectomized ram for detection of estrus.

LH-RH/FSH-RH Treatments. A decapeptide preparation of (pyro) Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly (NH₂) (Matsuo et al. 1971; Baba, Matsuo and Schally, 1971) was used in this study. This synthetic preparation was chemically pure as indicated by amino acid analysis and exhibited maximum biological potency as determined in ovariectomized, estrogen-progesterone (OEP) pretreated rats when compared with pure natural porcine LH-RH/FSH-RH (AVS-77-33 # 215-269) which has been thoroughly characterized by Schally et al. (1971). LH-RH/FSH-RH at a concentration of 50 μg/ml was placed in solution with acidified saline (0.01M acetic acid, 0.14M sodium chloride). Enough LH-RH/FSH-RH was put into solution at one time to conduct the entire experiment and this preparation was stored at 4°C between treatment periods. Treatments consisted of a single intramuscular (im) injection of either 50 μg LH-RH/FSH-RH or an equivalent volume of acidified saline. Ten ml blood collections were made via jugular puncture immediately preceding and at 30-min. intervals after the LH-RH/FSH-RH or saline injection for a subsequent 8 hours. The blood was allowed to clot and was stored for 24 hr. at 4°C. Following centrifugation, serum was aspirated off and stored frozen until assayed. The treatments were repeated three times at 45-day intervals and were designated as early (May 7), mid (June 21) and late (August 5) anestrus. These times were chosen because Reeves and Ellington (unpublished data) found in 20 Columbia ewes that the mean time for the beginning of anestrus was April 9 and the end was September 3. Thus the mean length of anestrus was 148 days and mid anestrus was approximately June 21. Thus June 21 was the logical mid anestrus date on the basis of these data and its being the longest day of the year. In the present study early and late anestrus data were collected 45 days on each side of June 21 to avoid any early or late sexual activity that might occur if longer intervals had been used. Each ewe received the same treatment for all three stages of anestrus. Each ewe was laparotomized by the technique of Hulet and Foote (1968) 4 hr. prior to each treatment and 48 hr. post treatment to determine if ovulation had occurred.

LH Assay. All serum samples were assayed by a double antibody radioimmunoassay as described by Reeves et al. (1970). Ovine LH (LER-1056-C2) was used for iodination and NIH-LH-S16 was used as a standard.

Estrogen Assay. Sera from samples collected prior to the initiation of treatment at each stage of anestrus were assayed for total estrogen using the same antiserum and radioimmunoassay technique described by Nett, Holton and Estergreen (1973). This antiserum binds 17β-estradiol and also binds estrone to a lesser extent. Previous studies have indicated 17β-estradiol to be the primary estrogen secreted into the ovarian venous circulation of the ewe (Short, McDonald and Rowson, 1963; Lindner, Sass and Morris, 1964; Moore et al., 1969). Since the circulating level of estrogens in anestrous ewes is very low, no attempt was made to fractionate these estrogens. One milliliter of each serum sample was extracted and radioimmunoassayable estrogen determined using 17β-estradiol to construct the standard curve. These estrogen values were used as an estimate of total estrogen without subtracting the water-blank value.

Results

All anestrous ewes treated with LH-RH/FSH-RH responded with a significant increase in serum LH by the first post treatment blood collection at 30 min., reached a peak between 1 and 2 hr. and returned to pretreatment levels by 4.5 to 6 hr. (figure 1). The time interval between LH-RH/FSH-RH injection and LH peak was not significantly altered by stage of anestrus (table 1). The mean time of the highest serum LH concentration after LH-RH/FSH-RH treatment for all stages of anestrus was 1.6 hours.

The concentrations of total serum estrogens before treatment were not significantly different between stages of anestrus (table 1). The magnitude of peak LH concentrations were not significantly affected by the stage of anestrus (table 1). The mean peak serum LH concentrations were higher (P < .01) in the LH-RH/FSH-RH treated early, mid and late anestrous groups (70.8 ± 16.7, 64.8 ± 13.2,
93.6 ± 20 ng/ml, respectively) than the mean basal levels in the saline treated groups (1.8 ± 0.44, 2.1 ± 0.30, 1.1 ± 0.14 ng/ml, respectively). When total serum estrogen concentrations before LH-RH/FSH-RH treatment were compared with peak serum LH concentrations after LH-RH/FSH-RH treatment, the correlation coefficient was \( r = -0.243 \) and was not significant (\( P > 0.01 \)). The mean value of water-blank in the estrogen assay was 2.8 ± 0.7 pg/ml with a 95% confidence interval ranging 1.3 to 4.3 pg/ml. The interassay coefficient of variation based on 11 separate determinations was 16%. These values compare favorably to those reported by Nett et al. (1973) and the minimum detectable amount of estrogen in this assay was 4 pg.

One ewe out of five ovulated after LH-RH/FSH-RH treatment at each of the three stages of anestrus (table 2), while none of the ewes ovulated after saline treatment during the three stages of anestrus studied. None of the ewes exhibited estrus during the three periods of anestrus.

**Discussion**

The mean peak height of the serum LH found after LH-RH/FSH-RH treatment at all three stages of anestrus 70.8, 64.8 and 93.6 ng/ml, is similar in magnitude to the preovulatory LH levels in serum of cycling ewes (Geschwind and Dewey, 1968; Niswender et al., 1969; Scaramuzzo, Caldwell and Moor, 1970; Reeves et al., 1970). None of the anestrous ewes including those which ovulated exhibited estrus after LH-RH/FSH-RH treatment. The lack of estrus in the ewes which did ovulate can be interpreted from the classical experiments showing that ovulation without estrus could be induced in anestrous ewes by the injection of pregnant mares serum gonadotropin (Cole and Miller, 1933; Dutt, 1952). It has been demon-

---

**Table 1. Mean Total Serum Estrogen Concentration Before Treatment, Peak Serum LH Concentration and Time of Peak Serum LH Following a Single Injection of LH-RH/FSH-RH in Ewes at Three Stages of Anestrus**

<table>
<thead>
<tr>
<th>Stages of anestrus</th>
<th>Serum estrogen concentration (pg/ml)</th>
<th>Peak serum LH (ng/ml)</th>
<th>Time of LH peak (hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE (n)</td>
<td>Mean ± SE (n)</td>
<td>Mean ± SE (n)</td>
</tr>
<tr>
<td>Early (May 7)</td>
<td>8.0 ± 1.4 (5)</td>
<td>70.8 ± 16.7 (5)</td>
<td>1.7 ± 0.2(5)</td>
</tr>
<tr>
<td>Mid (June 21)</td>
<td>8.4 ± 0.9 (5)</td>
<td>64.8 ± 13.2 (5)</td>
<td>1.3 ± 0.1(5)</td>
</tr>
<tr>
<td>Late (August 5)</td>
<td>7.1 ± 0.3 (5)</td>
<td>93.8 ± 20.0 (5)</td>
<td>1.8 ± 0.1(5)</td>
</tr>
</tbody>
</table>

*a* 17β-Estradiol Standard.  
*b* NIH-LH-S16.
strated that a source of progesterone either exogenous or endogenous (corpus luteum) is necessary prior to ovulation before symptoms of estrus will occur in the ewe (Robinson, 1954). Considerable variations within and between animals were noted in serum LH levels after LH-RH/FSH-RH treatment in the present study. Each ewe may have an endogenous cycle occurring during anestrus which cannot be seen by any overt phenomena such as estrus since ovarian follicles grow but do not ovulate. Thus all ewes may not be in a similar reproductive state as is usually assumed.

It has been suggested previously (Reeves et al., 1971 a,b) that estrogen levels can change pituitary responsiveness to LH-RH/FSH-RH. However, endogenous estrogen levels before treatment in the present study were not correlated with response to LH-RH/FSH-RH and cannot explain the large within and between animal variation in response to LH-RH/FSH-RH noted in this study. It is also possible that as endogenous estrogen levels were measured only at one point in time, their influence on subsequent response to LH-RH/FSH-RH may not have been adequately evaluated. It should also be noted that in the present study, the circulating levels of estrogens in the anestrous ewes were very low and were measured at the lower limit of assay sensitivity. Thus, lack of significant correlation between pretreatment endogenous estrogen levels and response to LH-RH/FSH-RH may not indicate a lack of estrogen effect.

Beck and Reeves (1973) treated ewes with 17β-estradiol at the identical stage of anestrus as used in the present study and found no significant differences in serum LH response between stages of anestrus. However, Domanski and Kochman (1968) found that ewes treated with ovine hypothalamic extracts during mid and late anestrus responded differently. Also, 40% of the latter authors’ ewes ovulated during mid anestrus while a majority ovulated during late anestrus. In the present study a low but equal percent of ewes ovulated at all three stages of anestrus. The discrepancy between Domanski and Kochman’s (1968) data and the present study may be due to various factors such as differences in preparations, modes of administration, dose administered, duration of administration or breed of sheep. They infused a natural extract into the pituitary for 5 hr. daily for 3 days while in this study the synthetic material was injected as one dose intramuscularly. Thus with a longer treatment period it may be reasonable to assume that follicular development may be accelerated and thus facilitate ovulation rate. However, in the present study there were no significant differences in pituitary responsiveness to LH-RH/FSH-RH at three different times during anestrus as noted by increased serum LH concentration or ovulation rate after a single im injection.

<table>
<thead>
<tr>
<th>Stage of anestrus</th>
<th>No. of ovulations</th>
<th>LH-RH/FSH-RH</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early (May 7)</td>
<td>1/5</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Mid (June 21)</td>
<td>1/5</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Late (August 5)</td>
<td>1/5</td>
<td>0/5</td>
<td></td>
</tr>
</tbody>
</table>

*aOvulation was detected by presence of corpus luteum.

Table 2. Ovulation in Anestrous Ewes After Treatment with LH-RH/FSH-RH in Acidified Saline

**Literature Cited**


SERUM LH IN ANESTRUS EWES


