Ovarian Response to Exogenous Hormones in Six-week-old Lambs

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Abstract

Crossbred lambs 5–6 weeks old were treated with human chorionic gonadotrophin (hCG) (500 or 1500 i.u.) alone, hCG plus pregnant mare serum gonadotrophin (PMSG) (1000 or 2000 i.u.), 1000 i.u. PMSG alone, or were untreated. PMSG alone and PMSG + hCG increased ovarian weight and uterine weight. PMSG alone stimulated growth and luteinization of follicles but PMSG + hCG induced ovulations and formation of corpora lutea. hCG alone did not change any of the characteristics which were measured. PMSG had a significant effect on the number of vesicular follicles but none of the treatments affected the number of growing follicles.

Introduction

Kennedy et al. (1974) reported that there were striking morphological changes in the ovaries of ewe lambs during the postnatal period. A significant increase in ovarian weight was accompanied by a marked proliferation and growth of vesicular follicles. The experiment reported here was designed to study the responsiveness of the follicles and the genital tract to exogenous gonadotrophins.

Mansour (1959) induced ovulation in 4- and 8-week-old lambs with pregnant mare serum gonadotrophin (PMSG) plus human chorionic gonadotrophin (hCG) but he studied very small numbers of animals and did not have adequate controls. Land and McGovern (1968) obtained ovulations in 9-week-old lambs with PMSG + hCG but they examined the ovaries only macroscopically.

Materials and Methods

Twenty-eight Border Leicester × Merino ewe lambs which were born in October were studied at Wellington, N.S.W. At 5–6 weeks of age the lambs were allocated at random to seven groups, each of four animals (Table 1). Group 1 remained untreated as a control. Between 1000 and 1030 h on day 0 the lambs in groups 4, 5 and 6 were injected intramuscularly with 1000 i.u. PMSG (Gestyl, Organon Laboratories) and in group 7 with 2000 i.u. PMSG. Lambs in groups 1, 2 and 3 were not injected with PMSG. Between 1030 and 1130 h on day 3 the lambs were injected intravenously with hCG (Pregnyl, Organon Laboratories) as follows: groups 2 and 5 with 500 i.u.; groups 3, 6 and 7 with 1500 i.u. Lambs were slaughtered between 1200 and 1600 h on day 4.

After slaughter the ovaries, uterus, cervix and Fallopian tubes were removed and fixed in Bouin’s fixative, rinsed in 50% (v/v) ethanol and stored in 70% (v/v) ethanol. The fixed organs were weighed, embedded in paraffin wax and sectioned at 6–8 μm thickness. The ovaries were stained with Gomori’s trichrome stain (Humason 1962), the reproductive tracts with PAS-Alcian blue (Culling 1974), and all were examined and measured as described by Kennedy et al. (1974). Quantitative data were analysed statistically to determine the significance of main effects (PMSG and hCG) and interaction. Because the design was an incomplete factorial, the method of fitting constants was used (Snedecor 1956).
Results

Means are presented in Tables 1 and 2. The ovaries of all control lambs contained large numbers of small, growing and vesicular follicles. The average diameter of the largest vesicular follicle in control ovaries was 2.7 mm.

Table 1. Mean organ weights and heights of the uterine epithelium of 5-6-week-old lambs treated with hCG and PMSG

<table>
<thead>
<tr>
<th>Group number:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>hCG (i.u.)</td>
<td>0</td>
<td>500</td>
<td>1500</td>
<td>0</td>
<td>500</td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td>PMSG (i.u.)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>2000</td>
</tr>
<tr>
<td>Ovarian weight (mg)</td>
<td>489&lt;sup&gt;a&lt;/sup&gt;</td>
<td>930&lt;sup&gt;b&lt;/sup&gt;</td>
<td>689&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2648&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1829&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3450&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1786&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uterine weight (mg)</td>
<td>2098&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2834&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2533&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6367&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3891&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4422&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5048&lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>Height of uterine epithelium (μm)</td>
<td>16.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>39.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.3&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
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Table 2. Mean numbers of follicles in ovaries of 5-6-week-old lambs treated with hCG and PMSG

<table>
<thead>
<tr>
<th>Group number:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG (i.u.)</td>
<td>0</td>
<td>500</td>
<td>1500</td>
<td>0</td>
<td>500</td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td>PMSG (i.u.)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>2000</td>
</tr>
<tr>
<td>No. of growing follicles per ovary</td>
<td>655</td>
<td>820</td>
<td>545</td>
<td>710</td>
<td>1405</td>
<td>580</td>
<td>950</td>
</tr>
<tr>
<td>No. of vesicular follicles per ovary</td>
<td>450&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>745&lt;sup&gt;b&lt;/sup&gt;</td>
<td>505&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>780&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>755&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>830&lt;sup&gt;a&lt;/sup&gt;</td>
<td>280&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>No. of follicles &gt;2.7 mm</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
<td>1.0</td>
<td>1.6</td>
<td>2.6</td>
<td>3.6</td>
</tr>
<tr>
<td>No. of ovulated follicles per ovary</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5.1</td>
<td>12.0</td>
<td>2.7</td>
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<tr>
<td>No. of luteinized follicles per ovary</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.8</td>
<td>1.0</td>
<td>1.2</td>
<td>2.2</td>
</tr>
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Treatment with hCG alone did not change any of the ovarian or uterine characteristics which were measured. Treatment with 1000 i.u. PMSG alone significantly increased ovarian and uterine weight compared with controls. The follicular response to PMSG alone varied between lambs—one lamb had ovaries which resembled those of the controls, and the other three lambs showed numerous vesicular follicles exceeding 2.7 mm in diameter but no follicle had ovulated. Hypertrophy of the uterine epithelium occurred in the two lambs in which there were luteinized follicles.

Treatment with PMSG+hCG (12 lambs) significantly increased mean ovarian weight, uterine weight and uterine epithelial height compared with control lambs. Only one of these lambs had ovaries which were similar to those of controls. In all others the ovaries contained numerous ruptured follicles and follicles greater than 2.7 mm in diameter, and the uterine epithelium had hypertrophied. Luteinized...
follies were present in 5 of these 12 lambs. The ovulatory response varied between lambs, the range in number of ruptured follicles per animal being 2–24. Lambs which received 2000 i.u. PMSG and 1500 i.u. hCG showed recently ovulated follicles and also corpora lutea which had apparently formed 1–2 days earlier, i.e. before the hCG had been given.

Analysis of variance of the data showed a significant interaction between PMSG and hCG \((P<0.01)\) for ovarian weight and uterine weight. The effect of PMSG was significant \((P<0.01)\) for the height of the uterine epithelium and the number of vesicular follicles, although there were significant differences between only two means when numbers of vesicular follicles in treatment groups were compared by Tukey’s test (Snedecor 1956).

**Discussion**

The ovaries of the control lambs were similar in appearance to those of the 4-week-old lambs described by Kennedy *et al.* (1974).

Mansour (1959), in a study of 9-week-old lambs, reported that treatment with PMSG alone did not induce ovulations. However, Land and McGovern (1968) reported that both recently ovulated follicles and mature corpora lutea were present 20 h after the injection of hCG which followed PMSG. We made a similar observation in the lambs which received 2000 i.u. PMSG and 1500 i.u. hCG. In these the corpora lutea appeared to be 1 or 2 days old and therefore must have formed from ovulations which were induced by PMSG before the hCG had been given. We conclude that PMSG alone, when given in sufficient quantity, can induce ovulations in very young lambs.

In our study, 1000 i.u. PMSG alone stimulated follicular development and luteinization of follicles, without ovulation. Turnbull *et al.* (1977) reported that luteinization of all follicles exceeding 3.5 mm in diameter was occurring in adult ewes 18 h after they had been treated with 750 i.u. hCG which had been given 48 h after treatment with 600 i.u. PMSG.

The enlargement of the uterus and hypertrophy of the uterine epithelium indicated that the stimulated follicles produced oestrogen. Uterine hypertrophy occurred in lambs treated with PMSG in which there were luteinized, but not ovulated, follicles. The production of oestrogen was substantial because the increase in the height of the epithelium in the PMSG + hCG-treated lambs was greater than the increase seen between dioestrus and metoestrus in adult ewes (Restall 1966), and was greater than that found by Raeside and Lamond (1956) in adult anoestrous ewes treated with 800 i.u. PMSG. Moor *et al.* (1973) have shown that short-term exposure to PMSG stimulates oestrogen secretion by both normal and atretic follicles greater than 2 mm in diameter. Since all follicles in the lamb which are greater than 1 mm in diameter show some signs of atresia (Tassell *et al.* 1978), PMSG must stimulate atretic follicles to secrete oestrogen. Trounson *et al.* (1977) have shown that the capacity of ovarian follicles from PMSG-primed, 10–16-week-old lambs to secrete oestrogen was similar to that of adult ewes. In their study follicles were greater than 3 mm in diameter when explanted. Peters *et al.* (1975) recently concluded that PMSG in immature mice did not increase the number of large follicles that developed but changed the balance between healthy and atretic follicles by preventing or delaying atresia. In our study PMSG increased the size of follicles, indicating that it either prevented atresia and allowed follicles to continue to grow, or promoted growth.
The addition of hCG to 1000 i.u. PMSG diminished the increase in uterine weight seen after 1000 i.u. PMSG alone (Table 1). The animals which received hCG ovulated and therefore would have been secreting progesterone. Progesterone inhibits oestrogen-induced growth of the uterus in the rat and other species by blocking replenishment of cytoplasmic oestrogen receptor (Hsueh et al. 1975). Furthermore, LH inhibits ovarian oestrogen secretion in the sheep (Moor et al. 1975). Thus the diminished growth of the uterus in those animals which received hCG and ovulated may have been due to the anti-oestrogenic effect of progesterone secreted by the corpus luteum, or to an effect of hCG, analogous to that of LH, in turning off oestrogen secretion.

The number of vesicular follicles seen in the lambs which had received 2000 i.u. PMSG followed by 1500 i.u. hCG was even less than that seen in the controls (Table 2). Turnbull et al. (1977) observed atresia in most medium-sized follicles of adult ewes given hCG after PMSG, and they related this to the wave of follicular atresia which in many species follows the pre-ovulatory LH surge. As this group of lambs which received 2000 i.u. PMSG was the only group which showed evidence of ovulation in response to PMSG alone, being the only group in which mature corpora lutea were seen, a decrease in vesicular follicle numbers may have been caused by follicular atrophy being induced by endogenous LH secretion.

PMSG and hCG are glycoproteins which are claimed to have both FSH-like and LH-like activities, PMSG being more similar to FSH and hCG being more similar to LH (Cole 1969). Our failure to obtain a response with hCG alone, even at a dose of 1500 i.u., suggests that the FSH-like activity present in this dose is inadequate for follicular development in the lamb. However, the study shows that stimulation with FSH (PMSG) followed by LH (hCG) can induce pre-ovulatory growth, ovulation and steroidogenesis in 6-week-old lambs. Plasma levels of LH and FSH in 6-week-old lambs are comparable with levels in anoestrous and dioestrous adults (Foster et al. 1975; Tassell et al. 1976) and pituitary content of these hormones is substantial. Thus it is likely that the ovarian responses obtained in the present study were due to augmentation of endogenous gonadotrophic activity by the exogenous hormones.

References


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