Ovarian Response to PMSG Treatment in Ewes Immunized against Oestradiol-17β


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Abstract
The effects of active immunization against oestradiol-17β on the ovarian response to pregnant mare serum gonadotrophin (PMSG) was investigated in Merino ewes. Immunized (79) and control (41) ewes were synchronized with intravaginal sponges, given either 750 or 1500 i.u. PMSG and then mated to rams or inseminated laparoscopically with fresh diluted semen. All control ewes mated naturally exhibited oestrus and 40 out of 41 control ewes ovulated. The ovulation rate was higher in the controls receiving 1500 i.u. PMSG than in those ewes which received 750 i.u. PMSG (10.2 v. 3.3).

Immunization against oestradiol-17β resulted in antibody titres varying from 100 to more than 100 000 in plasma taken 1–4 days after mating. The ovarian response increased significantly in the lowest titre group (100–1000) in conjunction with stimulation with 1500 i.u. PMSG. In these ewes the ovulation rate increased over controls (16.7 v. 10.2) as did the total ovarian response, which includes follicles greater than 10 mm diameter (22.3 v. 11.1). The total ovarian response was also increased in those ewes given 750 i.u. PMSG which had titres in the 1000–10 000 and 10 000–100 000 range, but this was not accompanied by significant increases in the ovulation rate. In general, the higher titre levels (>1000) were correlated with decreases in the proportion of ewes showing oestrus and ovulating and in the embryo recovery rate. The 1500 i.u. PMSG treatment group with the highest titres (>10 000) also showed a significant drop in the ovulation rate as compared to the 1500 i.u. PMSG controls.

Introduction

Gonadotrophins are responsible for follicular growth in several species of mammal, including sheep (Bindon et al. 1979) but the processes determining ovulation rate remain unclear (Scaramuzzi and Radford 1983). Administration of follicle stimulating hormone (FSH) or pregnant mare serum gonadotrophin (PMSG) increases the ovulation rate apparently by decreasing the proportion of large antral follicles which become atretic (Mauleon and Mariana 1977).

Active or passive immunization of sheep against various ovarian steroids have been shown to increase ovulation rates (Scaramuzzi 1976; Scaramuzzi et al. 1977, 1980, 1981). It has already been shown that controlled steroid immunity can form the basis of a commercial treatment for increased lambing percentages (Scaramuzzi et al. 1983). This technique and that of administration of gonadotrophins may act independently to give an additive ovarian response (Hoskinson et al. 1982). If so, then the use of embryo transfer in domestic animals, particularly cattle (Seidel 1981), may be facilitated by using a combination of these two methods to increase the supply of embryos.

The present experiment was designed to evaluate the effect of active immunization against oestradiol-17β on the superovulatory response of sheep to two different levels of PMSG. Recovery and fertilization rates following both natural and artificial methods of breeding were also evaluated in these sheep.
Materials and Methods

Experimental Design

Seventy-nine mature Merino ewes were used to assess the influence of two different methods of immunization against oestradiol-17β on the ovarian response to two levels of PMSG, either 750 or 1500 i.u. of Folligon (Intervet Australia Pty Ltd). Ewes were immunized with the aim of producing a range of antibody titres. Forty-one control ewes (group 1) were not immunized but received either dose of PMSG. The experiment was carried out in five replicates of 24 ewes with breeding occurring between 6 December 1983 and 10 January 1984.

Immunization

The antigen was synthesized by conjugating 17β-oestradiol-6-(O-carboxymethyl)oxime to human serum albumin by the dimide method in phosphate buffer–tetrahydrofuran (Lindner et al. 1972).

Various levels of anti-oestradiol antibody around ovulation were produced by using two immunoadjuvants and two injection regimes. Groups 2 and 3 were given the antigen (1 mg/ewe) in a solution (3 ml) of 5% (w/v) diethylaminoethyl-dextran (pH 7·5) (Cox and Wilson 1976), group 2 receiving only a primary injection and group 3 a booster 28 days following the primary. Groups 4 and 5 were given the antigen (1 mg/ewe) in an emulsion (3 ml) of Freund’s complete adjuvant, group 4 receiving only a primary injection and group 5 a booster 28 days following the primary. The primary immunization was given intramuscularly (1 ml to each hind leg) and subcutaneously (1 ml distributed over six dorsal sites). All primary injections were given 6 weeks before mating was due to occur.

The determination of antibody titres was similar to the method described by Abraham (1974). Blood samples were collected at the time of egg recovery and the plasma was stored at −20°C until assayed. Plasma was diluted serially with sodium/potassium phosphate buffer (0·05 M, pH 7·4) containing gelatin (0·1%), sodium chloride (0·9%) and sodium azide (0·1%). Radiolabelled oestradiol-17β (185 Bq, 10 pg) in 50 μl phosphate buffer was added to each dilution to make a final volume of 1·0 ml. The mixture was kept at 4°C (16 h) and dextran-coated charcoal added (0·1 ml consisting of 2% (w/v) decolourizing charcoal (Ajax Chemicals Pty Ltd) suspended in phosphate buffer containing 0·1% (w/v) dextran T-70 (Pharmacia [South Seas] Pty Ltd). The antibody titre is defined as the dilution of antisera which bound 50% of the radiolabelled oestradiol-17β available and is expressed as the reciprocal. The between-assay coefficient of variation for antibody titre determination was 13·8%.

Oestrus Synchronization and Insemination

All ewes were given intravaginal progestagen sponges containing 60 mg medroxyprogesterone acetate (Repromap, Upjohn Pty Ltd) for 14 days. Forty-eight hours before sponge removal half the total number of ewes in each group received 750 i.u. PMSG as a single i.m. injection and the remainder 1500 i.u. Paired groups of 12 ewes were started on synchronization treatment simultaneously; each group was divided among 10 treatments (i.e. five immunization × two PMSG dose levels) with the two control subgroups containing two ewes each and the other subgroups one. One group of each pair was bred naturally by fertile rams (1 : 4 ram : ewe ratio) bearingaddle harnesses and crayons (Sire Sine, Hortico Aust. Pty Ltd), starting 24 h after progestagen withdrawal. The other group was bred by intra-uterine insemination 24 h after progestagen withdrawal following which they were exposed to vasectomized rams (1 : 4) bearing harnesses and crayons. Semen was freshly collected and diluted with 1 vol. Dulbecco’s phosphate-buffered saline, with approximately 10⁶ spermatozoa being injected into each uterine horn. Naturally mated ewes were checked twice daily for raddle marks which were taken as indications of oestrous response. Data were not collected for those bred artificially.

The ovarian response was examined following slaughter of the ewes at least 36 h after oestrus was recorded or up to 5 days after progestagen withdrawal. A record was kept of the number of ovaulations, large (>10 mm diameter) follicles and numbers of eggs recovered. Fertilization was assessed by embryo cleavage or, for one-cell eggs, by the presence of sperm tails on the zona pellucida. Data were analysed using x² and, where indicated, one-way analysis of variance or t-test.

Results

The titre ranges of each group of sheep, apart from controls (<50) overlapped considerably. Thus five groups—control, A, B, C and D—were reformed based on
successive orders of magnitude of reciprocal titre ranges (Table 1). Ewes were evenly spread over groups A, B and C with eight of the total of 79 immunized ewes having titles in excess of 100 000.

**Oestrus and Ovulation**

Oestrus, observed only for mated ewes, ranged from 100% occurrence in controls through 90, 31, 27 to 0% for groups A–D. This decrease was significant ($P < 0.001$) irrespective of the PMSG treatment. Likewise the proportion of ewes ovulating declined significantly ($P < 0.01$) from control values as the oestradiol antibody titre increased (Table 1). At no stage were there any differences between oestrous responses at either level of gonadotrophin stimulation.

**Table 1. Ovarian responses, egg recoveries and fertilization rates following injection of 750 and 1500 i.u. PMSG in control ewes and ewes immunized against oestradiol-17β**

Values are means ± s.e.m.

<table>
<thead>
<tr>
<th>Group</th>
<th>Reciprocal titre range</th>
<th>No. of ewes</th>
<th>No. ovulating (%)</th>
<th>O.R.</th>
<th>Total ovarian response</th>
<th>No. of eggs recovered</th>
<th>F.R. (%)</th>
<th>No. of fertilized eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>750 i.u. PMSG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>No titre</td>
<td>20</td>
<td>20 (100)</td>
<td>3.3±0.5</td>
<td>3.7±0.5</td>
<td>54</td>
<td>87.0</td>
<td>2.35</td>
</tr>
<tr>
<td>A</td>
<td>100–1000</td>
<td>9</td>
<td>9 (100)</td>
<td>5.0±1.2</td>
<td>5.3±1.2</td>
<td>38</td>
<td>92.1</td>
<td>3.89</td>
</tr>
<tr>
<td>B</td>
<td>1000–10 000</td>
<td>16</td>
<td>5 (31.3)</td>
<td>6.0±2.1</td>
<td>5.5±0.7</td>
<td>20</td>
<td>80.0</td>
<td>1.00</td>
</tr>
<tr>
<td>C</td>
<td>10 000–100 000</td>
<td>9</td>
<td>2 (22.2)</td>
<td>3.0±2.0</td>
<td>6.6±1.3</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>&gt;100 000</td>
<td>6</td>
<td>0 (0)</td>
<td>—</td>
<td>5.0±0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1500 i.u. PMSG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>No titre</td>
<td>21</td>
<td>20 (95.0)</td>
<td>10.2±1.8</td>
<td>11.1±1.8</td>
<td>127</td>
<td>68.5</td>
<td>4.14</td>
</tr>
<tr>
<td>A</td>
<td>100–1000</td>
<td>12</td>
<td>8 (75.0)</td>
<td>16.7±2.9</td>
<td>22.3±2.0</td>
<td>83</td>
<td>31.3</td>
<td>2.17</td>
</tr>
<tr>
<td>B</td>
<td>1000–10 000</td>
<td>13</td>
<td>4 (69.2)</td>
<td>10.7±2.2</td>
<td>14.6±1.9</td>
<td>53</td>
<td>52.8</td>
<td>2.15</td>
</tr>
<tr>
<td>C</td>
<td>10 000–100 000</td>
<td>12</td>
<td>6 (50.0)</td>
<td>3.6±1.7</td>
<td>14.3±1.8</td>
<td>9</td>
<td>100</td>
<td>0.75</td>
</tr>
<tr>
<td>D</td>
<td>&gt;100 000</td>
<td>2</td>
<td>0 (0)</td>
<td>—</td>
<td>9.0±0.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

A Ovulation rate of ewes ovulating. Values with different superscripts differ significantly ($P < 0.05$).

B Of all ewes: number of ovulations + follicles >10 mm diameter. Values with different superscripts differ significantly ($P < 0.05$).

C Fertilization rate.

D Per ewe treated.

The ovulation rates of those ewes ovulating were similar for all groups that were injected with 750 i.u. PMSG. However, following treatment with 1500 i.u. (Table 1), ewes within the titre range 100–1000 experienced an increase in ovulation rate (ANOVA, $P < 0.02$). Further increases in titre eroded this effect and at the titre range of 10 000–100 000 a significant reduction from control occurred ($t$-test, $P < 0.05$).

Comparison of ovulation rates of PMSG-treated groups within each titre range indicated that only for control and group A were the responses to the larger dose of gonadotrophin greater than those to the smaller (ANOVA, $P < 0.002$).

**Total Ovarian Response**

There were no differences between immunized animals for total ovarian response to treatment with 750 i.u. PMSG (Table 1); however, groups B and C were elevated with respect to the controls (ANOVA, $t$-test, $P < 0.02$). Treatment with 1500 i.u.
resulted in the lowest titre group (A) having a significantly elevated response over all other groups (ANOVA, \( P < 0.006 \)) (Table 1). This treatment also resulted in higher total ovarian responses for each immunization group than those achieved in response to 750 i.u. (\( t \)-test, \( P < 0.01 \)).

**Recovery Rates**

Data were initially sorted into those ewes from which eggs were recovered and those from which no recoveries were made and analysed by \( \chi^2 \). There was no effect of the method of insemination on the proportion of ewes from which eggs were recovered. However, there was an overall decrease in the proportion of ovulating ewes from which eggs were recovered as titre levels increased, but the decrease was only significant in the group treated with 1500 i.u. PMSG (\( P < 0.001 \)).

The recovery rates obtained for ewes from which eggs were recovered were less when 1500 i.u. PMSG were injected, irrespective of whether uterine (48.7 \( \pm \) 73.7%) or natural insemination (62.4 \( \pm \) 84.5%) was used (\( P < 0.001 \)). Less eggs were also recovered from ewes artificially inseminated than from mated ewes (55.5 \( \pm \) 67.0%, \( P < 0.005 \)). There were significant downward trends associated with increases in titre for the mated group at both levels of gonadotrophin stimulation which resulted in a combined decrease from 78.4 to 39.1% (\( P < 0.0001 \)). This trend was also evident when groups inseminated by either method were considered together (67.0 \( \pm \) 41.9%, \( P < 0.02 \)).

**Fertilization Rates**

The proportion of ewes from which no fertilized eggs were recovered, as assessed by failure to cleave and the absence of spermatozoa on the zona pellucida, was similar between treatments with respect to the method of insemination, the dose of PMSG and the level of antibody achieved.

The method of insemination did not affect the overall fertilization rates (69.4% intra-uterine \( \pm \) 60.2% mated, \( P > 0.05 \)) but the higher dose of PMSG led to a decrease (84.5 \( \pm \) 55.1%, \( P < 0.0001 \)) which was consistent for intra-uterine or mated groups. Although values for subgroups showed significant variation within insemination and gonadotrophin treatments, there were no trends established with respect to antibody titres (Table 1).

**Yield of Embryos**

While the data concerning the number of fertilized eggs obtained per ewe treated were not suitable for analysis, it can be seen from Table 1 that no combination of treatments yielded more embryos than did 1500 i.u. PMSG given to control animals. No embryos were obtained from ewes with titres greater than 100 000.

**Discussion**

This study found there were effects of the level of anti-oestradiol antibody in the plasma of sheep on the yield of embryos obtained in response to two dose levels of the gonadotrophin, PMSG. The oestrous and ovarian responses of ewes, plus the recovery and fertilization rates of eggs, were all considered to contribute to the yields of embryos. Although the titre ranges produced were a result of four different immunization techniques, the single treatment using dextran adjuvant (group 2) provided most of the animals allocated to the lowest titre group A. A primary plus booster injection of antigen
given with Freund's adjuvant (group 5) provided most of the ewes allocated to the highest titre group D.

It has been shown that active immunization of sheep against several of the steroids of reproduction will increase ovulation rate provided excessive titres are not reached (Scaramuzzi and Radford 1983). In the current experiments there was a decreased oestrous response associated with an increase in circulating antibody concentration such that no ewes with titres greater than 100,000 displayed oestrus, nor did they ovulate despite evidence of some follicular development. There was a concomitant decline in the proportion of ewes ovulating, indicating that the oestradiol produced by the follicles was being bound to such an extent that interference with the ovulating luteinizing hormone discharge occurred (Scaramuzzi et al. 1970). It is possible that by giving an ovulating agent such as human chorionic gonadotrophin or luteinizing hormone releasing hormone (Nancarrow et al. 1984; Quirke and Hanrahan 1975) advantage may be taken of the increased ovarian response that was exhibited by some groups of ewes, particularly group A after treatment with 1500 i.u. PMSG. This group was the only one which exhibited an increased ovulation rate over the controls at either level of exogenous gonadotrophin stimulation. However, the fertilization rate of this group was the lowest recorded; thus no increase in the yield of embryos was achieved. Titres above 1000 obviously had a negative effect on ovulation in sheep although the effect on the total ovarian response varied with the dose of PMSG used. However, the higher dose of PMSG always produced a higher ovarian response.

Intra-uterine insemination led to a lower recovery rate in ewes from which eggs were recovered, most likely through handling of the reproductive tract although there was no difference between this group and that which was mated in the incidence of zero recoveries. Increasing levels of antibody appeared to have negative effects on some treatment groups, e.g. the 1500 i.u. PMSG and mated groups, but these interactions present no logical explanation. As the proportion of ewes from which eggs were recovered also declined, then we conclude that the higher titres of anti-oestradiol antibodies have a depressing effect on the recovery of eggs which is perhaps a result of excessive neutralization of circulating oestradiol which interferes with fimbrial development and oocyte pick-up.

The proportion of ewes in which there was complete absence of fertilization was unaffected by any treatment. Despite previous demonstrations of the beneficial effects of intra-uterine insemination over natural service in gonadotrophin-stimulated ewes (Trounson and Moore 1974; Boland and Gordon 1978; Nancarrow et al. 1984), our present results showed no significant difference. There appears to be some conflict in the literature on the effects of excess oestrogen and in particular oestradiol-17β on sperm transport. Noyes et al. (1959), working with ovariectomized rabbits, noted that up to 1 μg/day of oestradiol increased the efficiency of sperm transport but that greater amounts appeared to be detrimental. Data for the ewe also suggest that excessive oestrogen is detrimental to sperm transport, fertilization or both (Robinson 1968; Moore 1982). This may be the cause of lower fertilization rates seen in the groups given 1500 i.u. PMSG. However, Hawk et al. (1978) showed that small quantities of oestradiol-17β given during oestrus could improve sperm transport in the ewe. We hypothesized that immunization would prevent the development of excessive plasma concentrations of oestradiol at the time of mating of sheep superovulated with PMSG and therefore might aid in sperm transport. Our results are equivocal concerning this question as there was a lower fertilization rate in response to greater PMSG stimulation.
but the higher levels of antibody did not redress this problem. Evans and Armstrong (1984) found decreased sperm numbers in the oviducts of ewes treated with PMSG although this did not appear to impair the rate of fertilization.

This work has shown that significant increases in ovulation rate and in total ovarian response to stimulation with a high (1500 i.u.) dose of PMSG can be achieved by treating ewes with oestradiol antigen to produce controlled, low titres of antibodies. If these titres rise above 1000 then adverse effects occur and no advantage is gained. Although these treatments gave no increase in the yield of embryos, attention to fertilization and recovery rates by introducing laparoscopic insemination (Killeen and Caffery 1982) may prove useful. Also, treatments which show greater repeatability in the production of lower (<1000) antibody titres combined with methods which induce a high proportion of follicles to ovulate may provide a practical approach to increasing the efficiency of superovulation. The further development of these embryos must also be considered in any assessment of the usefulness of this technique.

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References


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