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Effect of Anti-oestradiol-17B Antibodies on the Reproductive Response of Ewes Superovulated with PMSG

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Abstract

A field experiment was conducted to examine the effect of anti-oestradiol-17B antibody titre on the oestrous and ovulatory responses of ewes to low (600 i.u.) or high (1200 i.u.) doses of pregnant mare's serum gonadotrophin (PMSG). Merino ewes were treated with intravaginal sponges and were subsequently used as vehicle-treated controls or were immunized to produce reciprocal anti-oestradiol-17B antibody titres of <1000 or >1000. Ewes were then treated with PMSG and the incidence of oestrus and ovulation, ovulation rate, and yield of embryos recorded.

Treatment of immune ewes with 1200 i.u. PMSG resulted in both a higher proportion of ewes ovulating and a higher ovulation rate than in immune ewes treated with 600 i.u. (86% *v.* 67% and 13·4 *v.* 6·0 respectively).

As anti-oestradiol-17B titres increased there was a reduction in the proportion of ewes exhibiting oestrus. The proportion of ewes ovulating decreased as antibody increased in ewes treated with 600 i.u. PMSG but not in those treated with 1200 i.u., suggesting an increased positive feedback of oestradiol with high PMSG doses. Fertilization rates were highest at the lower PMSG dose (68% *v.* 42%) and increased with increasing titre. Overall, there was no increase in ovulation rate or in yield of embryos over control values from either low (<1000) or high (>1000) antibody titres.

Introduction

Immunization against the ovarian steroids oestradiol-17B (Scaramuzzi 1976) and androstenedione (Scaramuzzi *et al.* 1977), and treatment with exogenous gonadotrophins (Bindon and Piper 1982) result in an increased ovulation rate in the ewe. Hoskinson *et al.* (1982) using oestrone-immunized ewes found evidence that the effects of PMSG and oestrogen immunity may be additive. Subsequently, Boland *et al.* (1985) using oestradiol-immune ewes found that ovarian response was enhanced only in ewes with low titres of antibodies. In ewes with reciprocal antibody titres greater than 1000 there was a lower incidence of oestrus and ovulation and a lower ovulation rate in those ewes ovulating.

This study was designed to confirm that an increase in ovulatory response and yield of embryos could be obtained for PMSG superovulated ewes in which anti-oestradiol-17B antibody titres were induced within the optimum range (100–1000) defined by Boland *et al.* (1985) and to assess whether the technique is of practical use under field conditions.

Materials and Methods

Experimental Animals

Animals used in this experiment were Merino ewes of 6–8 years of age, and run at the Hay Field Station of the University of N.S.W. in the western Riverina. Both before and during the trial, ewes were run under paddock conditions on dry native pastures.

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Experimental Design

During April the ovulatory response to two levels of PMSG (600 i.u. and 1200 i.u.) in ewes treated to induce three titre ranges of anti-oestradiol-17B (reciprocal ranges of 0, <1000, >1000) was examined in 180 Merino ewes in a 3 × 2 factorial experiment.

Immunization Treatments

The immunogen was prepared by chemical conjugation of oestradiol-6-O-carboxymethyl oxime to porcine thyroglobulin in a mixed solvent procedure similar to that described by Lindner *et al.* (1972) but using tetrahydrofuran as the steroid solvent. Reactants and their proportions were: steroid acid (0·5 mM), ethyl dimethylaminopropyl carbodiimide hydrochloride (0·5 mM) and protein (0·5 μM). Conjugation occurred in phosphate buffer (0·1 M, pH 7·8) over 16 h at ambient temperature. After evaporation of the tetrahydrofuran at 40°C and lyophilization of the aqueous phase the product was used as immunogen. Each dose of vaccine consisted of immunogen (equivalent to 1 mg protein) dissolved in 5% diethylaminoethyl dextran (DEAE-dextran, 2 ml, pH 7·5) and this was given as a single subcutaneous vaccination in the neck region.

Two vaccination régimes were used in an attempt to produce both low (<1000) and high (>1000) antibody titres. Groups of 60 ewes received either a single vaccination 6 weeks before sponge removal (group 2) or a primary and a booster given 3 and 1 weeks, respectively, before sponge removal (group 3). Eighty vehicle-treated control ewes (group 1) received only the 5% DEAE-dextran adjuvant, given to equal numbers at either 6 weeks or 3 and 1 weeks before sponge removal.

The method described by Boland *et al.* (1985) was used to determine serum anti-oestradiol antibody titres on blood samples collected from the jugular vein at the time of sponge removal. Antibody titres were expressed as the reciprocal of the dilution of antiserum that bound 50% of the radio-labelled oestradiol-17B available in the assay.

Oestrus Control and Superovulation Treatment

All ewes were treated with progestogen sponges (Repromap, Upjohn Pty Ltd) for 13 days. PMSG (Folligon, Intervet Aust Pty Ltd) was given as either a 600 or 1200 i.u. dose by intramuscular injection 48 h before sponge removal. Immediately following sponge removal ewes were run with 10% harnessed entire Merino rams, and raddle marks were recorded at 12-hourly intervals for 96 h as indicative of the time of onset of oestrus.

Ewes were slaughtered at an abattoir 6 days after sponge removal and their ovaries and uteri collected. Within 10 h of slaughter the numbers of corpora lutea and unovulated follicles greater than 10-mm diameter (Boland *et al.* 1985) had been recorded. The uterus of each animal was flushed with saline and the numbers of ova recovered and cleaved were recorded. In most ewes the uterine flushings contained considerable suspended cellular material and difficulty was experienced in thoroughly searching these for embryos.

Statistical Analysis

The effect of immunization treatment on titre of antibody and on distribution of oestrus was analysed using a χ-squared test, while effects on ovulation rate and yield of embryos were subject to least squares analysis of variance procedures (Harvey 1960).

Results

Titre Levels

Both immunization treatments produced a wide range of individual antibody titre responses at the time of sponge removal (titre range 50–8000). However, a higher proportion of ewes in group 3 had antibody titres above 1000 than did ewes in group 2 [17/58 (29%) *v.* 5/60 (8%), $\chi^2_1 = 8{\cdot}5$; $P < 0{\cdot}01$].

For the purposes of further analysis the three groups were re-formed on the basis of individual ewe antibody responses falling in the ranges of nil, 1–1000 or 1000–8000, irrespective of vaccination treatment (Table 1).

Oestrus and Ovulation

As antibody titres increased, significant reductions in the proportion of ewes exhibiting oestrus were obtained ($\chi^2_1 = 27{\cdot}3$; $P < 0{\cdot}01$, Table 1). As well, the onset of oestrus was delayed in ewes with higher antibody titres (Table 1, $P < 0{\cdot}01$). A higher proportion of ewes treated with 1200 i.u. PMSG exhibited oestrus than did ewes treated with 600 i.u. PMSG (86% *v.* 67%, $\chi^2_1 = 8{\cdot}5$; $P < 0{\cdot}01$).

Table 1. Effect of anti-oestradiol-17B antibody titre on oestrous response of ewes treated with PMSG

Values with different superscripts differ significantly, $P < 0{\cdot}05$

Reciprocal titre range	Number of ewes		Mean time of onset of oestrus (hours after sponge removal)
	Treated	Oestrus	
0	58	56 (97%)^A	35 ± 1·5
0–1000	96	68 (71%)^B	48 ± 1·4
>1000	22	10 (46%)^B	61 ± 4·2
Total	176	134 (76%)	43 ± 1·6

Only in the low-PMSG-treated groups was there a significant reduction in the proportion of ewes ovulating as antibody titre increased (Table 2). A significantly higher proportion of ewes treated with 1200 i.u. PMSG ovulated than did ewes treated with 600 i.u. PMSG (90 *v.* 72%; $P < 0{\cdot}01$).

Table 2. Ovarian response of ewes immunized against oestradiol-17B to treatment with PMSG

Values with different superscripts differ significantly, $P < 0{\cdot}05$

Reciprocal titre range	Number of ewes		Least-square mean + s.e.	
	Treated	Ovulating	Ovulation* rate	Total ovarian response
		600 i.u. PMSG		
0	28	28 (100%)^A	4·9 ± 1·5	5·8 ± 1·5
0–1000	43	30 (79%)^B	4·5 ± 1·4	6·1 ± 1·6
>1000	16	4 (25%)^C	8·5 ± 3·9	10·5 ± 4·3
Total	87	62 (72%)	6·0 ± 1·5	7·5 ± 1·6^A
		1200 i.u. PMSG		
0	30	29 (97%)^A	14·8 ± 1·4	19·1 ± 1·6
0–1000	53	46 (87%)^A	14·0 ± 1·1	18·5 ± 1·3
>1000	6	5 (84%)^A	11·4 ± 3·4	17·2 ± 3·8
Total	89	80 (90%)	13·4 ± 1·3	18·3 ± 1·4^B

*Ovulation rate of ewes ovulating.

Ovarian Response

There was no significant effect of antibody titre on ovulation rate or on total ovarian response (corpora lutea plus large follicles); however, dose of PMSG significantly affected ovarian response. Ewes treated with 1200 i.u. PMSG had a significantly higher ovulatory and total ovarian response than did ewes treated with 600 i.u. PMSG ($P < 0{\cdot}01$). There was no interaction between antibody titre and PMSG dose on ovulatory or ovarian response.

Recovery and Fertilization Rates

While antibody titre had no effect on recovery rate of embryos it did affect the proportion of recovered ova that were fertilized (Table 3). Fertilization rates increased from 38% in controls to 54% ($\chi^2_1 = 15{\cdot}1$; $P < 0{\cdot}01$) and 66% ($\chi^2_1 = 12{\cdot}2$; $P < 0{\cdot}01$) in ewes with antibody titres below and above 1000 respectively.

A higher proportion of ova were recovered in ewes treated with 600 i.u. PMSG than in ewes treated with 1200 i.u. PMSG (58% *v.* 47% respectively; $\chi^2_1 = 10{\cdot}1$; $P < 0{\cdot}01$) and fertilization rates were higher (68% *v.* 42% respectively, $\chi^2_1 = 37{\cdot}5$; $P < 0{\cdot}01$).

Table 3. Production of embryos by ewes immunized against oestradiol-17B following treatment with PMSG

Values with different superscripts differ significantly, $P < 0{\cdot}05$

Reciprocal titre range	No. of ewes treated	Number of ova Recovered	Number of ova Fertilized	Number of embryos*
		600 i.u. PMSG		
0	28	86 (63%)	50 (59%)	2·0±0·6
0–1000	43	67 (50%)	50 (75%)	1·2±0·5
>1000	16	22 (65%)	19 (87%)	1·2±0·8
Total	87	175 (58%)	119 (68%)	$1{\cdot}5 \pm 0{\cdot}4^{A}$
		1200 i.u. PMSG		
0	30	189 (44%)	$54\ (29\%)^{A}$	1·8±0·6
0–1000	53	319 (50%)	$155\ (49\%)^{B}$	3·3±0·5
>1000	6	24 (43%)	$11\ (46\%)^{B}$	1·8±1·4
Total	89	532 (47%)	220 (42%)	$2{\cdot}3 \pm 0{\cdot}5^{B}$

*Mean number embryos per ewe treated (least-square mean + s.e.).

Yield of Emrbyos

Only the dose of PMSG had a significant effect on yield of embryos. Ewes treated with 600 i.u. PMSG yielded fewer embryos than did ewes treated with 1200 i.u. PMSG ($P < 0{\cdot}05$). Yield of embryos was unaffected by antibody titre.

Discussion

In immunized ewes not stimulated with gonadotrophins there is a reduction in unbound steroid, a reduction in negative feedback resulting in an increase in plasma FSH concentration and an increase in ovulation rate (Fairclough *et al.* 1976; Smith 1985). Ovulation rate responds in a curvilinear manner to increasing titre levels, with ovulation being inhibited at high antibody titres (Martin *et al.* 1979; Smith *et al.* 1983; Scaramuzzi and Radford 1983; Webb *et al.* 1984). In PMSG-treated oestradiol-immune ewes, Boland *et al.* (1985) observed similar increases in ovulation rate and total ovarian response to superovulatory (1500 i.u.) doses of PMSG in ewes with low (<1000) titres of anti-oestradiol-17B antibody. However, even with low levels of antibody, we observed a reduction in the incidence of oestrus and ovulation, with no change in ovulation rate of ewes ovulating.

Reasons for the differing results are not clear but in the present study the depression in the incidence of oestrus and ovulation in response to increasing antibody titre was partly overcome by increasing the dose of PMSG. This indicates an alteration in positive feedback effects due to higher doses of PMSG stimulating follicular activity in immune ewes resulting in an increase in the circulatory levels of biologically available oestrogens. However, in those ewes ovulating, the fact that no increase in ovulation rate was observed in the immunized

groups given in the lower dose of PMSG suggests that the concentration of unbound oestrogen in plasma was sufficient to maintain negative feedback control on FSH production by the pituitary gland (Radford, Nancarrow and Findlay 1978). In this experiment it would appear that lower concentrations of unbound oestrogen, which would result in increased FSH levels and increased ovulation rates (Smith 1985), were not obtained without inhibition of positive feedback necessary for oestrus and ovulation. Determination of free and bound oestrogen levels would be required to confirm this hypothesis.

Recovery rates of embryos were somewhat low compared with published values (Moore and Shelton 1962) possibly due to the difficulty experienced in locating embryos in the flushings of most ewes. The observation that fertilization rates increased with antibody titre and at the lower dose of PMSG is consistent with the observations of Robinson (1968) and Moore (1982) that sperm transport and hence fertilization are lowered by high levels of ciculatory oestrogens at the time of mating. This evidence is consistent with the increase in oestrus and ovulation being a result of higher plasma levels of biological oestrogen at the higher dose of PMSG.

While this work achieved its objective to produce low (<1000) levels of antibody titre, it has failed to show that the method is of any benefit in either ovulation rate or in the production of embryos in superovulation programmes, as suggested by Boland *et al.* (1985).

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