Dose-response to a single injection of PMSG in Merino ewes carrying a double copy of the Booroola Fec^B gene

G H SHACKELL AND K L ISAACS

AgResearch, Invermay Agricultural Centre, P.O. Box 50034, Mosgiel, New Zealand.

ABSTRACT

A dose-response curve for ovulation rate in Merino ewes homozygous for the Booroola Fec^B gene was generated by injecting PMSG at dose rates increasing from 300-1200iu in 100iu increments (n=8-13 per group). PMSG was administered, at a concentration of 1000iu/ml in a saline carrier, as a single injection 15 days after the withdrawal of intravaginal progestagen sponges. An additional 20 control ewes were injected with 1ml of saline.

The mean lifetime ovulation rate (LO) was calculated for each ewe from data collected for up to three years prior to the study. In individual ewes, a "response" to PMSG was defined as an ovulation rate at least 4 standard deviations (flock mean SD 0.87) greater than LO. Using this arbitrary value, no animals responded at doses below 500iu and less than 50% responded at 500 and 600iu. Above 800iu 75-100% of ewes in each treatment group responded. When log ovulation rate was regressed against dose of PMSG there was a highly significant dose-response ($P<0.001$, RSD 0.512).

Using a subjective assessment of changes in ovarian morphology it was observed that ≥70% of ewes given 1000iu or more of PMSG showed over-stimulation of one or both ovaries. There was no apparent carry-over effect of PMSG at the oestrous cycle following treatment.

Keywords Booroola, Fec^B, PMSG, Dose-response.

INTRODUCTION

We have previously reported a simplified technique for increasing the ovulation rate of Merino ewes carrying a double copy (BB) of the Booroola Fec^B gene (Shackell and Isaacs 1991). The technique involved synchronising the animals with progestagen sponges, followed by a single injection of 500iu Pregnant Mare's Serum Gonadotrophin (PMSG) on day 15 after sponge withdrawal. There was no recording of mating activity following synchronisation and all animals were injected irrespective of whether or not they showed oestrus. The dose rate, which was chosen arbitrarily, increased the mean ovulation rate of the treated animals from 4.9 prior to treatment, to 8.3 (range 5-18) after treatment.

The aim of the present study was to generate a dose-response curve for ovulation rate in BB Merino ewes following a single injection of PMSG administered under this regime. The necessity to increase ovulation rate in these naturally highly fecund animals is most likely to be as part of an embryo transfer programme. Since harvesting of embryos from superovulated donors is therefore the prime objective, it is essential that treatment does not compromise embryo recovery rates. The occurrence of enlarged ovaries, unruptured follicles and haemorrhagic *corpora lutea*, all of which in our experience appear to adversely effect embryo recovery rates, is therefore undesirable. As dose rate of PMSG increases so does the likelihood of changes in the gross morphology of the ovaries. While embryo recoveries were not attempted in this study, ovarian appearance was monitored.

The objective of the study therefore, was to determine a dose-rate which gave an acceptable mean ovulation rate response with little or no ovarian trauma, and yet maintained the simplicity of a single injection of PMSG.

METHODS

Merino ewes (N=116) all of known BB genotype, were synchronised during the breeding season with intravaginal sponges containing 45mg fluorogestone acetate (Chronogest; Intervet). On day 15 following sponge withdrawal, ewes (n=8-13 per group) were injected with PMSG (Folligon; Intervet), at a concentration of 1000iu/ml in a sterile saline carrier. Ten dose rates (300iu to 1200iu in 100iu increments) were used. An additional 20 control ewes were injected with 1ml of sterile saline. Ovarian gross morphology (number of *corpora lutea* (CL), number and size of unruptured follicles and the general appearance of the ovaries) was determined by laparoscopy 6 days after PMSG treatment.

Since all ewes in the study were part of the Tara Hills Booroola flock, ovulation rates recorded during routine procedures over the two or three years prior to the experiment were available. These records were used to calculate a life-time mean ovulation rate (LO) for each ewe. As an arbitrary estimate, a response to PMSG (R) was defined as an ovulation rate (OR) higher than LO by at least four times the mean flock standard deviation, ie:

$$R = (OR > LO + (4 \times 0.87)) \quad [1]$$

Ewes were laparoscoped again 34 days after treatment and this observation compared to LO to check for the occurrence of carry-over effects on subsequent ovulation rate.

To assess the extent of ovarian stimulation each ovary was scored as either (i) normal in appearance, or (ii) abnormal, which included ovaries with numerous unruptured follicles and/or haemorrhagic CL's and/or were enlarged to such an extent that we considered embryo recovery rates were likely to be impaired.

STATISTICAL ANALYSES

Ovulation rate data were log transformed to stabilise variance, and the transformed data regressed against dose rate, with LO then added to the model. To test the notion that excessive stimulation may occur as a function of dose, ovary score data were analysed as a generalised linear model with binomial error distribution and logit link function (Nelder and Wedderburn, 1972).

RESULTS

Response to PMSG

There was a highly significant effect ($P<0.001$) on the proportions of animals responding, as defined by equation [1], as dose of PMSG increased (see Table 1). PMSG did not significantly alter ovulation rate in any animals when given at 300 or 400iu. Ovulation rate responses were recorded in up to 70% of individuals at dose rates from 500-700iu while doses of 800iu and above resulted in more than 70% of animals responding.

There was no apparent residual effect of PMSG on ovulation rate when the ewes were routinely laparoscoped at the end of the subsequent cycle. Mean ovulation rate of the treated animals 34 days after treatment was not different from their mean LO (5.04 *vs* 5.03; SED 0.13). Table 1 also shows mean LO and mean ovulation rate following the study for each treatment group.

TABLE 1 The proportion of BB Merino ewes responding to increasing doses of PMSG, their mean lifetime ovulation rate (LO), mean ovulation rates (OR) following treatment (and range), the proportion with ovarian over-stimulation and post treatment ovulation rate.

Dose PMSG	Proportion Responding	LO	OR (Range)	Propn with excessive stimulation	Post-treatment OR
0	0	5.2	6.2 (4-10)		6.1
300	0	5.8	5.0 (3-7)	0	5.9
400	0	5.0	5.7 (0-8)	0	4.5
500	0.31	5.0	9.8 (4-24)	0.08	5.4
600	0.10	4.7	6.9 (0-15)	0.10	5.0
700	0.63	4.7	9.1 (3-13)	0.13	4.3
800	0.80	5.2	12.7 (4-28)	0.30	4.7
900	1.00	4.7	16.5 (11-27)	0.25	5.4
1000	0.78	5.4	12.0 (6-16)	0.33	5.4
1100	0.73	4.7	13.6 (1-30)	0.73	4.5
1200	1.00	5.2	19.9 (10-40)	0.70	5.2

Ovulation rate

There was a highly significant dose-response of ovulation rate to PMSG ($P<0.001$) as shown in Fig. 1. The regression equation (with standard errors) is given by:

$\log(OR) = 1.42 (\pm 0.146) + 0.0011 (\pm 0.0002)X$; ($RSD = 0.512$, $R^2(adj) = 28.2\%$)

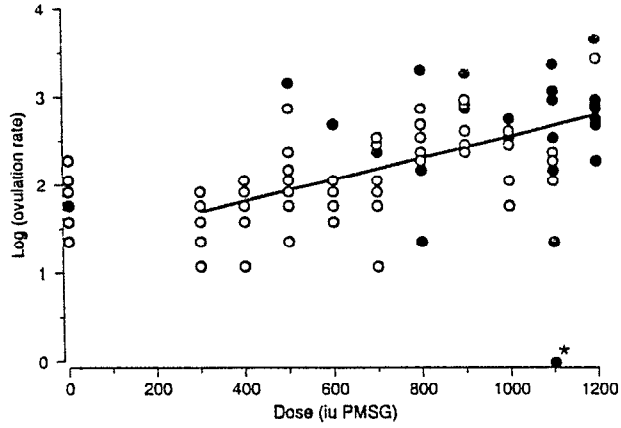
where X= dose of PMSG.

One ewe in the 1100iu group had excessively stimulated ovaries and an ovulation rate of 1 (see Fig. 1). If this ewe is omitted from the analysis the equation changes to:

$\log(OR) = 1.35 (\pm 0.124) + 0.0012 (\pm 0.00015)X$; ($RSD = 0.433$, $R^2(adj) = 40.3\%$.

There was considerable variability in ovulation rate between individuals given the same dose. The mean ovulation rate and the range for each treatment group is shown in Table 1. The inclusion of LO in the above regression model, did not account for any further variation, suggesting that the response to PMSG administration was not related to natural ovulation rate.

FIGURE 1 Dose response of BB Merino ewes to a single injection of PMSG given at the time of sponge withdrawal.



○ Animals with normal ovaries
● Animals with excessively stimulated ovaries

* Note one ewe which performed atypically and alters regression equation when omitted from data (see text).

Ovarian morphology

One ewe in the control group had one ovary which appeared abnormal at the time of laparoscopy. This phenomenon is occasionally seen in BB Booroola ewes during routine recording of natural ovulation rate (G H Shackell *pers comm*), and is generally noted as the occurrence of large, unruptured follicles. Among the treated animals in the present study, there was a strong positive relationship between dose rate and the incidence of abnormal ovarian morphology. In ewes given doses below 500iu there were no animals with abnormal ovaries. At dose rates between 500 and 700iu one or both ovaries were scored as abnormal in 8-13% of animals. Dose rates of 800-1000iu resulted in 25-33% of ewes showing some change in ovarian morphology. At doses above 1000iu one or both ovaries were classified as being other than normal in ≥70% of animals. The proportion of ewes at each dose rate which were recorded as over-stimulated is shown in Table 1.

DISCUSSION

This study confirms our previous report that a single injection of PMSG at sponge removal increases ovulation rate in BB Merino ewes. Furthermore, as dose rate increases so does ovulation rate, but this is accompanied by a parallel increase in the occurrence of abnormal changes in ovarian morphology.

The ovulation rates recorded in these BB ewes were higher than would be expected from non-carrier ewes given similar doses. Kelly *et al.*, (1983/84) have shown that injecting Booroola Merinos (classified as either B+ or ++ on ovulation rate history) with PMSG at sponge withdrawal, increased ovulation rate. In that study there was a linear response to PMSG at lower doses although the mean ovulation rate of ++ ewes at the highest dose (1000iu) was greater than would have been predicted. Nevertheless the ovulation rate of ++ ewes given 1000iu PMSG (7.2) was still lower than that recorded for BB ewes (12.0) given the same dose in our study. Although there are similar numbers of non-

atretic follicles in ewes with and without the Booroola gene, follicles mature at a smaller diameter in animals with the gene (McNatty *et al.*, 1985). This suggests that the putative Booroola gene effect is mediated by an enhanced sensitivity of granulosa cells to pituitary hormones, and is the most logical explanation for the higher ovulation rate responses achieved in BB Merino ewes with comparatively low doses of PMSG.

Merino ewes with a double copy of the Fec^B gene are naturally highly fecund. Natural ovulation rates in the flock used for this study have a mean of about 5.0. Individuals have been recorded as having up to 10 CL's at any one observation, although this phenomenon is rare and not highly repeatable (K L Isaacs, *pers comm.*). A single injection of 500-700iu of PMSG to BB ewes on day 15 after withdrawal of progestagen sponges resulted in up to 60% showing an ovulation rate response. The mean ovulation rate of these groups increased to about 4.5 above mean lifetime ovulation rates recorded prior to the study. At these lower dose rates, ovarian morphology appeared normal. However, this study also recorded an increase in the occurrence of changes in gross ovarian morphology concomitant with increasing ovulation rate as dose rate increased. A dose of 1100 or 1200iu PMSG resulted in mean ovulation rates of 13-20 with individual ewes shedding up to 40 ova. At these high dose rates mean ovulation rate increased by up to 14.7 compared to the lifetime mean. However, a high proportion (up to 70%) of ewes showed over-stimulation on one or both ovaries.

While the mean ovulation rate response is better at the higher dose rates, the associated changes in ovarian morphology suggest that embryo recovery rates are likely to be compromised. Therefore if this simplified technique is to be used in BB Merino ewes, it is recommended that dose rates do not exceed 1000iu PMSG. A dose rate of about 700iu is likely to be the most satisfactory, since any increase beyond this level also increases the likelihood of excessive stimulation.

ACKNOWLEDGEMENTS

We gratefully acknowledge the help of I.C. Scott with laparoscopic examinations, and the staff at Tara Hills High Country Research Station for technical assistance. Dr R.P. Littlejohn provided valuable help with statistical analyses of the data.

REFERENCES

Kelly R.W.; Owens J.L.; Crosbie S.F.; McNatty K.P.; Hudson N. Influence of Booroola Merino genotype on the responsiveness of ewes to Pregnant Mares Serum Gonadotrophin, luteal tissue weights and peripheral progesterone concentrations. *Animal reproduction science*, **6:** (1983/84) 199-207.

McNatty K.P.; Henderson K.M.; Lun S.; Heath D.A.; Ball K.; Hudson N.L.; Fannin J.; Gibb M.; Kieboom L.E.; Smith P. (1985) Ovarian activity in Booroola x Romney ewes which have a major gene influencing their ovulation rate. *Journal of reproduction and fertility* **73:** 109-120.

Nelder J.A.; Wedderburn R.W.M. (1972) Generalized Linear Models. *Journal of the Royal Statistical Society* **A 135:** 370-384.

Shackell G.H.; Isaacs K.L. (1991) A simplified MOET technique for ewes carrying a double copy of the Booroola Fec^B gene. *Proceedings of the New Zealand Society of Animal Production* **51:** 107-109.