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## A simplified MOET technique for Merino ewes carrying a double copy of the Booroola $Fec^B$ gene

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### ABSTRACT

As part of a programme to increase the number of homozygous ( $Fec^B Fec^B$ ) replacements available in the Booroola Merino flock at the Tara Hills Research Station a MOET technique was utilised. Because ewes of BB genotype are difficult to superovulate, with predictable results, using conventional treatment regimes, a simplified programme aimed at enhancing the natural ovulation rate was used. Thirty eight ewes were treated for 10-11 days with intravaginal sponges. The ewes were then injected with 500 iu of PMSG on day 15 after sponge withdrawal and joined with entire rams for 5 days. Sixty six percent of ewes were marked by the ram and the mean ovulation rate of these was 8.3 (range 5 - 18). Embryo recovery rate was 93.4% following retrograde flushing of the fallopian tubes. Fertilisation rate was 68% and 5.3 embryos were transferred per donor operated (range 0 - 11). The ewes were remated and lambed naturally. By using a combination of MOET and remating, 24 donor ewes produced a mean of 3.3 lambs weaned compared to 1.4 lambs weaned by their naturally mated flock mates.

**Keywords** Booroola, ovulation rate, MOET, PMSG.

### INTRODUCTION

The Booroola  $Fec^B$  gene is a major gene(s) (Davis *et al.*, 1982, a & b; Piper and Bindon, 1982) which controls ovulation rate in ewes. Davis and Kelly (1983) defined criteria by which ewes can be assigned  $Fec^+ Fec^+$  (++; ovulation rate <3),  $Fec^B Fec^+$  (B+; ovulation rate 3-4) or  $Fec^B Fec^B$  (BB; ovulation rate >5) genotypes. A closed BB Merino flock is maintained at the Tara Hills High Country Research Station. As well as providing a source of animals for studies on the Booroola gene within New Zealand, genetic material from this flock has been exported to Canada, Israel, Scotland and the USA. The maintenance of this unique flock relies on survival of 8 family lines to ensure that inbreeding is minimised.

Because of its high ovulation rate the Booroola has the ability to produce litters of lambs (Davis *et al.*, 1984). It is well established that as litter size increases, lamb birthweights, and hence survival rates, decrease (Hinch *et al.*, 1983). At Tara Hills over the past 7 years BB ewes have had a mean ovulation rate of 5.0, but only 1.77 lambs born and 1.20 lambs weaned per ewe mated.

In an attempt to improve on these figures, and to bolster some family lines which were becoming low in numbers, a decision was made to use Multiple Ovulation Embryo Transfer (MOET) to increase the number of lambs born to those family lines in the 1990 breeding season. Logistical constraints required use of a simple technique which would give a modest lift in ovulation rate and good quality transferrable embryos.

Earlier attempts at this station to superovulate Booroola ewes with conventional dose rates (eg 1000-1200 iu) of Pregnant Mares' Serum Gonadotrophin (P.M.S.G.) have proved inappropriate. Often the ovaries show large, apparently cystic, follicles and haemorrhagic corpora lutea (CL); as well fertilization and embryo recovery rates may be compromised (G.H. Shackell, unpublished). It has therefore been standard practice to utilize the natural ovulation rate of around 5.0 when collecting embryos from Booroola ewes.

### DEVELOPMENT OF METHOD

Late in the 1987 breeding season it was necessary to collect embryos from BB ewes in the Tara Hills Booroola flock. A decision was made to give each ewe 500 iu of

P.M.S.G. to ensure oestrous activity. The dose rate was based on responses reported by Kelly *et al.* (1983) for B+ ewes and was expected to give little or no lift in ovulation rate for BB ewes. To optimise fertilisation rates donor ewes were not mated until the second cycle after synchronisation. The ewes were treated for 12 days with intravaginal sponges containing 70 mg. Medroxyprogesterone acetate (Repromap; Upjohn). After sponge withdrawal the ewes were joined with teaser rams and tup marks recorded twice daily. On day 13 after ewes were marked by the teaser ram they were injected with 500 iu P.M.S.G. (Folligon; Intervet). Ewes not marked were injected on day 15 after sponge withdrawal. A further group of donor ewes were injected with P.M.S.G. at sponge withdrawal. Following P.M.S.G. ewes were single sire mated as they showed oestrus. The results are shown in Table 1.

The ovulation rates were much higher than anticipated with some ewes shedding 20 or more ova. The excellent result from the low-dose injection of PMSG 15 days after sponge withdrawal lead to a decision to exploit the simple methodology for future applications.

**TABLE 1** Ovulation rate response (OR), and percentage of ova recovered and fertilised related to time of P.M.S.G. injection.

P.M.S.G.	n	OR ± SD	% Ova Recovered	% Ova Fertilised	No. embryos/ donor
sponge withdrawal	16	6.3±2.8	46.4	68.3	2.0
13 days post oestrus	33	8.7±5.4	64.4	76.6	4.3
15 days post sponge withdrawal	15	15.8±9.5	49.1	69.6	5.4

## EXPERIMENTAL

In 1990 donor ewes were synchronised with Fluorogestone acetate sponges (Chronogest; Intervet, Australia) for 10-11 days and injected with 500 iu PMSG on day 15 after withdrawal (day 25 or 26). No monitoring of oestrus immediately following sponge withdrawal was done. Recipient ewes were treated with progesterone devices (CIDR.G; Carter Holt Harvey, Hamilton, New Zealand) to cycle at the same time as the donors were being mated. During the mating period all

ewes were checked for oestrus at 0800 and 1600 h daily.

The results of the surgery programme are shown in Table 2. Of 38 treated donors 25 were mated and subsequently operated. Ovulation rate of donor ewes was assessed at laparotomy 4.5 days after onset of oestrus. The ovaries showed no signs of cystic follicles or haemorrhagic CL. Ova were recovered by retrograde flushing of the fallopian tubes. The rams used were not proven and poor fertilization rate (63%), especially from one ram, reduced the number of embryos available for transfer.

**TABLE 2** Results of surgery programme (mean values per donor).

Number of	Total	Treated (n=38)	Operated (n=25)
CL	207		8.6*
ova recovered	186 (93%)	4.9	7.8
Embryos transferred	126 (63%)	3.3	5.3
lambs born	60	1.6	2.5
lambs weaned	57	1.5	2.4

\* 1 ewe with no fallopian tubes excluded from all subsequent data.

Embryos were transferred to recipient ewes in pairs into a uterine horn ipsilateral to a CL(s) unless pedigree requirements necessitated transfer as a single. Each recipient was laparoscoped and following examination of the ovaries the appropriate tip of the uterine horn was exteriorised via a 2-3 cm incision. Embryo(s) were aspirated into the uterine horn, the tissue washed with saline, returned to the abdominal cavity and the incision closed. Embryo survival rates were lower than normally achieved (G.H. Shackell, unpublished).

Following surgery all donor ewes which had been operated received Prostaglandin (Estrumate; Coopers Animal Health) and were remated. During the programme ewes in the BB flock not required for MOET were naturally mated. Table 3 compares the mean number of lambs weaned by a combination of MOET and subsequent remating or after natural mating alone. The 24 donor ewes from which ova were flushed resulted in 61 lambs born following transfer, and of these 58 were weaned. Twenty-one donors were present at lambing. Thirteen of these lambed and produced a

further 34 lambs born, of which 22 were weaned. Thus the 24 ewes weaned a mean of 3.3 lambs. The combination of MOET and remating resulted in individual ewes producing from 0 to 11 lambs for the season. In contrast the part of the flock which were naturally mated produced 1.9 lambs born and 1.4 lambs weaned per ewe mated.

**TABLE 3** Mean number of lambs from operated and naturally mated ewe.

Breeding System	OR ± SD	Lambs Born	Lambs Weaned
MOET (n = 24)	8.3±3.4	2.5	2.4
Donors remated*		1.4	0.9
Total MOET group*		3.9	3.3
Natural (n = 167)	5.5±1.4	1.9	1.4

\* 8 of 21 ewes present at lambing were dry

### CONCLUSION

This simple, single injection technique induced an increase in ovulation rate in BB ewes. By combining this increase with embryo transfer and remating donor ewes the mean number of lambs weaned per donor was about 1.4 more than from naturally mated contemporaries. Higher survival rate of transferred embryos would improve this result. Currently studies to define the most appropriate dose of P.M.S.G. to give maximum ovulatory response in Booroola ewes are being undertaken.

### ACKNOWLEDGEMENTS

We gratefully acknowledge the help of I.C. Scott, J.R. Land, M. Jansen and staff at Tara Hills. Also to Dr A.J. Allison whose insistence that we use P.M.S.G. in a programme where it was considered by some as unnecessary, has resulted in a serendipitous, simple, but very useful technique.

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