OESTRADIOL IMPLANT REDUCES LITTER SIZE IN THE BOOROOLA MERINO

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SUMMARY

The litter size of homozygous Booroola Merino ewes results in high perinatal mortality. One subcutaneous implant of crystaline oestradiol in silastic tubing in heterozygous Booroola Merino ewes decreased ovulation rate from 3.18 ± 0.15 (n = 39) to 2.03 ± 0.14 (n = 35), and decreased litter size (5 - 8 weeks pregnant) from 2.49 ± 0.13 (n = 39) to 1.47 ± 0.12 (n = 32), providing a useful management tool. In homozygous Booroola Merino ewes ovulation rate decreased from 4.11 ± 0.28 (n = 18) to 2.11 ± 0.28 (n = 18). Two implants prevented ovulation in about half of the animals and only 9 of 38 such heterozygous ewes had viable foetuses at 7 weeks.

Keywords: Booroola, oestradiol, ovulation, embryo, mortality

INTRODUCTION

The homozygous Booroola Merino has a mean ovulation rate of 4.2 and a lambing rate of 2.5 (range 1 - 7) (Bindon 1984). This prolificacy of the Booroola is due to a single gene (FecB gene), which affects only the reproductive rate by increasing the ovulation rate (Bindon and Piper 1986). The Booroola provides a resource for research into the physiology and genetics of reproduction. With respect to usage by industry, crosses with other Merinos produce a 45 - 56% increase in litter size at birth and a 16 - 37% increase in the number of lambs weaned (Bindon 1984).

There is a need to maintain a homozygous Booroola flock as there are currently no markers available, either genetic or physiological, and carriers of the FecB gene can be identified only by phenotypic effects in females (Montgomery *et al.* 1995). Embryonic mortality is increased because of the high ovulation rate with substantial wastage of potential embryos in ewes with ovulation rate greater than 3 (Hanrahan 1980; Bindon 1984). Lambs born in large litters are small, the main cause of their greater mortality (Owens *et al.* 1985), and require artificial lamb rearing. Additional husbandry care is required for lambing ewes and this also increases costs (Piper and Bindon 1991). A technique that decreases ovulation rate, and hence litter size, without altering the genetic composition of offspring, would be beneficial for both economic and welfare reasons. Webb *et al.* (1992) and Wright and Hariadi (1994) have shown that implants of oestradiol decrease the ovulation rate in a variety of sheep breeds. We investigated the effects of 1 or 2 implants on the ovulation rate, fertility and litter size in order to use this technique to maintain a flock of Merino ewes homozygous for the FecB gene. We were encouraged in this by finding that others had, on an *ad hoc* basis, used implants to reduce litter size in a few Booroola ewes (R. Webb, personal communication).

MATERIALS AND METHODS

Animals and experimental design

Experiment I Merino ewes (3yr old) that had been shown to carry 1 copy of the FecB gene were available after use in a previous experiment which found no effects of immunization with synthetic peptides mimicking the structures of FSH and LH receptors or Mullerian inhibiting hormone (M. A. Hillard, N. Jones, T. O'Shea and R. Webb, unpublished results). They were allocated randomly, within sire groups and previous treatments, to receive either an empty capsule (n=39) or 1 (n=40) or 2 (n=38) oestradiol capsules on Day 11 after the second (Day 0) of two injections of 0.125 mg cloprostenol (Estrumate, Pitman-Moore, Sydney). For mating, rams fitted with Sire-sine harnesses and marking crayons were added on Day 25. Marks were recorded until the ewes were killed on Days 87 and 88 at a pet food abattoir when the reproductive tracts were collected for examination at the laboratory, and the implants checked. Ovulation rate, defined as the number of CL per ewe in ewes with at least 1 CL, was determined by laparoscopy on Days 8 and 25 and by dissection on Day 87 or 88. Foetuses were dissected out on Day 87 or 88 and crown-rump distance measured with calipers. Any remnants of degenerated foetuses were recorded.

Experiment 2 Merino ewes (n=54), aged 4 years, carrying 2 copies of the FecB gene, were randomly allocated to receive (Day 0) either an empty implant, or 1 or 2 oestradiol capsules. Onset of oestrus was synchronised by progesterone-impregnated intra-vaginal devices (C.I.D.R. Type G, AHI, Plastic Moulding

Co., Hamilton, NZ) between Days 10 - 20. At removal on Day 20 a single injection of cloprostenol was given to ensure luteolysis in these prolific animals. Numbers of corpora lutea were counted by laparoscopy on Day 28, with a repeat examination of unovulated sheep on Day 37.

Implants

The capsules were prepared as described by Karsch *et al.* (1973). One end of a 4.2cm length of Silastic Medical-Grade tubing (0.132in i.d. x 0.183in o.d., catalogue number 601-335 Dow Corning, Midland, Michigan) was sealed with a 0.5cm plug of silicone rubber (Silastic adhesive, Dow Corning), crystalline 17B-oestradiol (Steraloids Inc, Wilton, NH) was packed to a height of 3.2 cm, and the open end sealed as before. Before use the capsules were incubated in distilled water at 37°C for 3 days to prevent an initial peak in plasma oestrogen (Karsch *et al.* 1973). A trocar and cannula were inserted subcutaneously on the inner surface of the forelimb in the wool-free area in the axilla. The capsules were implanted through the cannula some 10cm from the puncture wound which was closed with a Michel clip in some ewes with 2 capsules.

Statistical analyses

Foetal crown-rump data were examined by AOV for unequal group size (Snedecor and Cochran 1967). Other data for the sheep with 1 or 2 oestradiol implants were compared with that for the controls by t-tests for groups with unequal numbers or by Chi-square analysis. Proportions of ewes that did not ovulate or carry a viable foetus after insertion of 1 or 2 oestradiol capsules were compared by Chi-square analysis.

RESULTS

Experiment 1

As there were no effects from treatments carried out in the previous year, data for such subgroups were combined to produce Table 1. At slaughter 1 implant had leaked, 1 ovary was not recovered and 1 uterus was not recorded from the group with 1 oestradiol capsule. Data affected were excluded. One oestradiol implant decreased OR after insertion for 14 days by 1.15 ($P < 0.001$) and at slaughter by 1.37 ($P < 0.001$), decreased litter size at 5 - 8 weeks by 1.02 foetuses ($P < 0.001$), early loss by 0.36 ($P < 0.05$), but increased returns to oestrus (i.e. mating cycles) by 0.15 ($P < 0.05$). Two implants decreased OR after 14 days by 1.58 ($P < 0.001$) and at slaughter by 1.35 ($P < 0.001$), litter size at 5 - 8 weeks by 1.16 ($P < 0.001$), and increased episodes of mating by 0.44 ($P < 0.001$).

Table 1. The effects (mean values ± SE) of oestradiol capsules on ovulation rate (OR), number of mating cycles, pregnancy, and litter size inheterozygous Booroola Merino ewes

Parameter	Control	1 oestradiol capsule	2 oestradiol capsules
Shorn liveweight (Kg)	32.62 ± 0.45 (39)[A]	32.65 ± 0.53 (38)	32.83 ± 0.62 (38)
OR pretreatment	3.54 ± 0.16 (39)	3.44 ± 0.17 (39)	3.57 ± 0.16 (37)
OR post treatment	3.18 ± 0.15 (39)	2.03 ± 0.14** (35)	1.60 ± 0.18** (20)
OR at slaughter	3.10 ± 0.14 (39)	1.73 ± 0.14** (33)	1.75 ± 0.25** (12)
Mating cycles	1.03 ± 0.03 (35)	1.18 ± 0.06* (38)	1.47 ± 0.08** (36)
Interval to successful mating (weeks)	5.34 ± 0.06 (35)	5.35 ± 0.16 (32)	5.55 ± 0.35 (10)
Litter size (viable foetuses)	2.49 ± 0.13 (39)	1.47 ± 0.12** (32)	1.33 ± 0.17** (9)
CL minus number of viable foetuses	0.62 ± 0.13 (39)	0.26 ± 0.09* (31)	0.73 ± 0.38 (11)
Ewes with viable foetus	39 of 39	32 of 38*	9 of 38**

[A] Group size in parentheses.

* Treated group significantly different from control animals ($P < 0.05$

**Treatment group significantly different from control animals ($P < 0.001$).

The interval from Day 0 to a successful mating was not changed by 1 or 2 implants but there was greater variability. Fewer ewes carried at least 1 viable foetus when implanted with 1 ($P < 0.01$) or 2 (P c 0.001) oestradiol capsules than did the control sheep.

Foetal crown-rump length (CR) was apparently increased by 1 or 2 implants, eg in sheep killed 7.3 weeks after a successful mating, foetal CR was greater ($P < 0.005$) in treated ewes (8.53 ± 0.10cm, n=25) than in control ewes (8.08 ± 0.09, n=21). However, when data for foetuses of sheep pregnant for 6.9 or 7.3 weeks were assigned to groups with 1 or 2 or 3 viable foetuses (this included 60 of the 76 ewes with viable foetuses plus a mating record), AOV for groups of unequal size showed that treated ewes had foetuses with CR (8.07cm) not significantly different from those of control ewes (7.93cm). The foetuses were significantly larger ($P < 0.025$) at 7.3 (8.41cm) than at 6.9 (7.59cm) weeks. There was a significant ($P < 0.025$) linear decrease in CR with increasing litter size (I foetus, 8.42cm; 2 foetuses, 7.82cm; 3 foetuses, 7.76cm).

More ewes with 2 implants than ewes with 1 implant had not ovulated post-treatment (17 of 37 vs 4 of 39, $P < 0.005$) and at slaughter (12 of 37 vs 4 of 37, P c 0.005), so that fewer had a viable foetus at slaughter (9 of 38 vs 32 of 38, $P < 0.005$).

Experiment 2

Ovulation rate was less ($P < 0.001$) in the ewes with either 1 (2.24 ± 0.26, n=17) or 2 (2.00 ± 0.37, n=10) oestradiol implants than in the control ewes (4.11 ± 0.28, n=18). These data include results from 3 ewes with 2 implants that had ovulated at the repeat laparoscopy. As in experiment 1, most of the ewes that had 2 implants and did not ovulate showed a marked increased tone of the tract, with the uterus being tightly coiled despite the absence of follicles with diameter > 2mm. More ewes with 2 implants than ewes with 1 implant did not ovulate (8 of 18 vs 1 of 18, $P < 0.01$).

DISCUSSION

Oestradiol implants reduced ovulation rate and consequently litter size, albeit with some loss of fertility even with 1 capsule. This variability of response was also seen in the time to conception. It is possible that a slightly smaller capsule would remove the infertility but may leave ewes with > 3 ovulations. Two capsules per ewe are not suitable as, through the complete suppression of ovulation, they induced a much greater loss of fertility than did 1 capsule. There was no effect on foetal growth, except that due to the smaller litters. Smith *et al.* (1993) used embryo transfer to equalise litter sizes of foetuses with and without the Booroola gene. They observed that the FecB gene decreased foetal body weight at 40 days but not at 90 days gestation. In the current study most ewes were killed at times intermediate to these days. It is anticipated that 1 capsule will be adequate for several years. To test this the homozygous Booroola ewes will be reexamined in 1996 and then mated.

One capsule may result in a greater depression in ovulation rate in homozygous ewes, although experiment 2 was carried out later in the breeding season than experiment 1. If true, this would imply that the Booroola Merino is more sensitive to oestradiol as well as to inhibin as shown directly by Cummins *et* al. (1983), and indirectly by their greater response to immunisation with inhibin (McNatty *et al.* 1991). These are the 2 hormones that exert negative feedback control of FSH secretion during the follicular phase in sheep (Martin *et al.* 1988).

In conclusion, 1 oestradiol implant will decrease the litter size in homozygous ewes so the flock can be more easily managed. The genetic makeup of progeny will be unaltered.

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