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Evaluation of the feasibility of a juvenile MOET scheme in sheep

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ABSTRACT

The feasibility of a juvenile MOET (multiple ovulation and embryo transfer) scheme in sheep was evaluated in three trials using 6-7 month-old animals. Ovulation rate was not affected by the dose (1.3 vs 1.1) or the time (1.5 vs 0.9) of PMSG administration, but it was higher in adults than lambs (2.0 vs 1.15, $P < 0.05$) (Trial 1). Adults also showed a higher percentage of animals in heat (89 vs 60, $P < 0.05$) and a higher percentage of eggs recovered (87 vs 56, $P < 0.05$) or fertilised (82 vs 48, $P < 0.05$). In trial 2, a higher response was achieved with PMSG than FSH-P, Ovagen or PMSG + Ovagen (4.18 vs 1.78, 1.06 and 2.05, $P < 0.05$). GnRH did not affect the ovarian response to gonadotrophins (2.29 vs 2.25, $P < 0.05$). Embryo viability appeared higher in lambs treated with Ovagen alone. A combination of Ovagen + PMSG and with or without GnRH did not improve significantly ($P < 0.05$) the response compared to the administration of PMSG plus GnRH (2.3, 2.8, 2.2 and 2.8 vs 1.7) (Trial 3). The superovulatory treatments involved failed to induce satisfactory ovulatory responses in these prepubertal sheep.

Keywords Lambs, gonadotrophin, ovulation rate, embryo transfer.

INTRODUCTION

The combination of multiple ovulation and embryo transfer (MOET) has the potential for increasing the rate of progress in genetic improvement schemes. Through the use of MOET, selection could be more intense and the generation interval considerably reduced compared to normal reproduction rates (Land and Hill, 1975). The theoretical benefits of such methodologies have been assessed for cattle (Nicholas and Smith, 1983) and sheep (Smith, 1986). For sheep a juvenile MOET scheme seems most promising. Toro *et al.* (1988), calculated that the rate of genetic change for wool production could be more than doubled compared with schemes using normal reproduction rates, provided that the generation interval can be reduced to one year. The implementation of such schemes under practical conditions requires good embryo transfer rates and good embryo survival from 6-8 month-old ewe lambs.

Although MOET techniques are available for sheep and have been used for the last 40 years, there are no reports in the literature using such techniques on a large scale with ewe lambs. The main objective of the present studies was to evaluate the feasibility of a juvenile MOET scheme in sheep.

MATERIALS AND METHODS

A MOET programme was conducted in March-April of 1988, 1989 and 1990. The first study involved (as donors) 46 six to seven month-old ewe lambs of which 25 were from a Romney fleece weight selection flock (FW) and the remaining 21 were from the control line (C). An additional group of mixed age Romney ewes was included for comparison. Oestrus was synchronised using intravaginal sponges prepared in our laboratory, containing 40 mg medroxyprogesterone acetate and inserted for 12 days. Superovulation was attempted using 900 i.u. or 1200 i.u. of pregnant mare's serum gonadotrophin (PMSG; Heriot Developments Pty, Australia) injected intramuscularly at sponge removal or 24 h before sponge withdrawal. Rams aged 18 months were introduced (1 ram:4 donors) at sponge removal. The ewes were inspected in the morning and in the afternoon and mated animals were flushed by laparotomy for embryo recovery 5.5 days after onset of oestrus. The procedures used for embryo recovery and transfer were as described by Tervit and Havik (1976). Recipient ewes were synchronised in a similar way to the donors, but sponge removal was 12-15 h before withdrawal from the donor ewes to obtain a better

TABLE 1 Effect of gonadotrophin treatment on embryo recovery (Trial 2).

	LAMBS								EWES	
	T1 PMSG	T2 PMSG + GnRH	T3 FSH-P	T4 FSH-P + GnRH	T5 OVA GEN	T6 OVA + GnRH	T7 OVA + PMSG	T8 OVA + PMSG + GnRH	T1 PMSG	T2 PMSG + GnRH
No. treated	9	8	9	9	8	8	9	11	9	11
No. in oestrus (%)	8(89)	8(100)	7(78)	6(75)	3(37)	8(89)	8(73)	7(78)	11(100)	
No. flushed	8	7	6	6	4	1	7	8	9	11
Total corpora lutea (range)	27 (0-7)	44 (0-15)	18 (0-9)	14 (0-6)	15 (0-7)	2 (0-2)	20 (0-5)	21 (0-6)	59 (1-16)	68 (2-18)
CL/donor treated	3.0	5.5	2.0	1.6	1.9	0.3	2.2	1.9	6.6	6.2
% eggs recovered ^a	49	68	44	39	27	50	39	48	65	77
% eggs fertilised ^b	92	64	50	75	100	100	75	67	84	65
% viable embryos ^c	47	56	50	0	100	100	75	25	80	67

^a = (eggs recovered/No. flushed); ^b = (eggs fertilised/eggs recovered); ^c = (viable embryos/eggs fertilised)

degree of synchronisation. Recipients showing oestrus within 24h of that of the donor ewes were used for embryo transfer.

In the second year, 73 ewe lambs (39 FW and 34 C) in 8 groups, were treated with sponges as previously outlined and 2 days before withdrawal given gonadotrophins. The treatments were: 1200 i.u. PMSG (Intervet, Australia) (T1); T1+100 µg gonadotrophin releasing hormone given i.v. at onset of oestrus (GnRH: Intervet, Australia) (T2); 24 mg porcine FSH-P (Schering Corporation, USA) given twice daily for 3 d in a decreasing dose regime (T3); T3+100 µg GnRH (T4); 8 ml of ovine FSH (Ovagen, I.C.P., New Zealand) in 8 even injections every 12h (T5); T5 + 100 µg GnRH (T6); T1 + 2.5 ml of Ovagen given four days after sponge insertion (T7); T7 + 100 µg GnRH (T8). Donor ewes were either naturally mated or intra-uterine inseminated.

In the third year, 64 ewe lambs (34 FW and 30 C) in 5 groups were synchronised as before and two days before sponge removal the following treatments given: 1200 i.u. PMSG + 100 µg GnRH (T1); 8 ml Ovagen + 300 i.u. PMSG given immediately before first Ovagen injection (T2); T2 + 100 µg GnRH (T3); as for T2 but

using 500 i.u. PMSG (T4); T4 + 100 µg GnRH (T5). Donors were intra-uterine inseminated with fresh semen. The percentage of eggs recovered, fertilised and viable as well as the ovulation rate data were analysed by ANOVA techniques. Analyses of the percentages were carried out after Arcsin transformation and the ovulation rate after log₁₀ (ovulation + 1) transformation. Differences in the incidence of oestrus was tested using Chi-square analysis. Group means were compared using Duncan's multiple range test.

RESULTS

Trial 1

The analysis of the ovulation rate data did not show any significant effect of either the time of PMSG administration (0.9 vs 1.5, for PMSG given at sponge removal or one day before, respectively) or the dose of PMSG (1.3 vs 1.1, for 900 or 1200 i.u. PMSG, respectively) injected. However, ovulation rate was significantly affected ($P < 0.05$) by age of donor with ewes showing higher response than lambs (2.0 vs 1.1, respectively). There was also a significant effect of age

TABLE 2 Effect of gonadotrophin treatment on embryo recovery (Trial 3).

	LAMBS					EWES
	T1 PMSG + GnRH	T2 OVA + PMSG	T3 OVA + PMSG + GnRH	T4 OVA + PMSG	T5 OVA + PMSG + GnRH	T1 PMSG + GnRH
No. treated	13	13	13	13	12	13
No. in oestrus (%)	10(77)	12(92)	13(100)	11(85)	10(83)	10(77)
No. flushed	9	12	10	8	10	11
Total corpora lutea (range)	18 (0-4)	32 (0-5)	37 (0-8)	23 (0-5)	33 (0-5)	41 (0-15)
CL/donor treated	1.4	2.5	2.8	1.8	2.8	3.2
% eggs recovered ^a	37	51	46	47	44	42
% eggs fertilised ^b	100	69	71	50	50	67
% viable embryos ^c	33	42	60	50	50	75

a = (eggs recovered/No. flushed); b = (eggs fertilised/eggs recovered); c = (viable embryos/eggs fertilised)

of donor on the percentage of ewes showing heat (89 vs 60, $P < 0.05$) and the percentage of eggs recovered (87 vs 56, $P < 0.05$) or fertilised (82 vs 48, $P < 0.05$) for ewes and lambs, respectively.

Trial 2

A summary of the results is given in Table 1. Overall the ovulation rates recorded were higher than in the previous year, despite a severe outbreak of facial eczema. There was no significant effect of GnRH on the ovulatory response (2.25 vs 2.29, for animals with and without GnRH). Ovulation rate was significantly higher ($P < 0.05$) in PMSG treated animals compared to animals treated with pituitary gland preparations (4.18 vs 1.78, 1.06) (FSH-P, Ovagen). There was no significant effect of the gonadotrophin treatment on the percentage of animals detected in heat and the percentage of eggs recovered or fertilised. However, the percentage of viable embryos was significantly higher ($P < 0.05$) in lambs treated with Ovagen than with PMSG, Ovagen + PMSG and FSH-P (100 vs 51, 46 and 20, respectively).

Trial 3

A summary of the results is shown in Table 2. There was no significant effect of the gonadotrophin treatment on any of the variables studied. Around 80% of the animals showed heat and similar percentage of them were flushed. The range of ovulation rates was less in lambs than ewes. Although the percentages of eggs recovered and fertilised were similar in ewes and lambs (42 vs 37 and 67 vs 100, respectively), the percentage of viable embryos was considerably lower in lambs particularly in the group treated with PMSG (75 vs 33).

DISCUSSION

The superovulatory treatments used failed to induce a satisfactory superovulatory response in most of the animals. Overall, high variability in the ovulatory response was observed confirming general findings from superovulated ewes (Robinson, 1951; Evans and Robinson, 1980). This large variability in response has been referred as the major limiting factor in all embryo

transfer programmes (Mapletoft and Murphy, 1989). The improvement in ovulation rate after PMSG administration in trial 2, could partly be due to the different source of PMSG used. The ovulatory results with the pituitary extracts were poor in contrast to those following the use of PMSG. The better results with PMSG differ from reports where mature ewes (Armstrong and Evans, 1983; Monniaux *et al.*, 1983) and goats (Tervit *et al.*, 1986) have been treated. However, better results with PMSG were reported in cattle (Monniaux *et al.*, 1983). This could suggest that prepubertal sheep require higher levels of LH in the superovulatory regime to achieve superovulatory response since LH half life is longer in PMSG (McIntosh *et al.*, 1975). This is supported by the fact that lambs treated with Ovagen (which has a low LH content, <0.2%) gave the lowest ovulatory response. GnRH, injected at the onset of oestrus did not have a statistically significant effect but the tendency for ovulation rates to be higher was consistent, particularly in PMSG-treated ewe lambs. Further trials with GnRH may be justified.

The ovulatory response was not improved by the combination of Ovagen + PMSG. However Ryan *et al.* (1984) and McMillan and Hall (1991), combined PMSG with pituitary gland preparations and achieved higher ovulation rates in ewes than when the pituitary preparation was used alone.

Overall no significant difference between gonadotrophin treatments was observed on the proportion of animals showing heat and the percentage of eggs recovered or fertilised. However, a significantly higher percentage of viable embryos was found in ewe lambs treated with Ovagen. This is in agreement with reports associating low LH content with better embryo quality (Mapletoft and Murphy, 1989). Although strict comparisons can not be made between ewes and lambs, generally the responses were lower in lambs than in adult ewes.

The results from these trials show some of the difficulties in implementing a MOET programme in these prepubertal sheep, which might be associated with the breed being studied.

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