EGG TRANSFERS IN EXOTIC SHEEP

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SUMMARY

Fifty-one aged "exotic" ewes were superovulated with PMSG and eggs recovered 3 to 6 days after mating. One hundred and twelve eggs were transferred to 74 recipients. The donor hormone treatment resulted in precise synchronization of oestrus and satisfactory superovulation, but 41% of the donors had corpora lutea undergoing premature regression. Egg recovery was very low in these animals (28%) and this combined with a low egg fertilization rate (57%) resulted in only 112 eggs being transferred. The recipient performance was satisfactory and 57 lambs were born. The combined lambing performance of the donors post-surgery and their recipients showed that the number of offspring was increased in three of the four breeds in comparison with the previous season's performance of the donors. The limited success of the programme was largely due to problems associated with the donor hormone regime and to poor performance of the "exotic" rams.

INTRODUCTION

THE TECHNIQUE of egg transfer has been used to rapidly increase the number of progeny from individual sheep (Rowson and Adams, 1957) and groups of sheep (Moore and Shelton, 1962). This paper describes an egg transfer programme carried out to increase the number of progeny from "exotic" ewes at the Mana Island Quarantine Station.

MATERIALS AND METHODS

Fifty-one 6- to 9-year-old ewes (19 Finnish Landrace (F), 5 East Friesian (E), 20 Oxford Downs (OX) and 7 German White Headed Mutton (G)) from the "exotic" stock imported in 1972 were chosen as donors. The F and OX animals selected were those which had produced the fewest live female progeny in New Zealand while the E and G animals represented all the original animals present at the station. One hundred and eighty-nine 7-year-old Romney (R) ewes were made available as recipients.

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Hormonal Treatments and Management of Animals

Donors

Intravaginal (I.V.) sponges containing 50 mg "Cronolone" (Searle) were inserted for 14 days on either 18 or 22 February 1975. After sponge removal the ewes were run with harnessed vasectomized rams and oestrus recorded daily. Then, either 9 or 10 days after sponge removal, all ewes were injected intramuscularly (i/m) with pregnant mares' serum gonadotrophin (PMSG). The F donors received either 800 or 1 200 i.u., the E 1 200 i.u., the OX 1 600 or 2 000 i.u. and the G 1 500 i.u. Two days after PMSG the animals were injected i/m with 125 μg prostaglandin $F_2\alpha$ analogue (PGFA, I.C.I. 80996). The ensuing oestrus was first detected with vasectomized rams (ewes examined at approximately 4-hourly intervals) and the ewes were then placed with their allotted entire ram in a mating pen and observed to mate. They remained in the pen for approximately 24 hours.

Recipients

I.V. sponges containing 50 mg "Cronolone" were inserted for 14 or 15 days on 1 or 5 March 1975. When these were removed the ewes were run with harnessed vasectomized rams and oestrus recorded twice daily. The recipient hormone regime was programmed so that the recipients were expected in oestrus at the same time as the donors following treatment with PMSG and PGFA.

Surgical, Egg Recovery and Transfer Procedures

Prior to surgery all ewes were starved for 24 hours .The donors were anaesthetized with Nembutal (Abbott Laboratories) and the eggs recovered in sterile sheep serum from either the oviducts 3 to 4 days after mating using the technique of Hunter *et al.* (1955) or from the uterus 5 to 6 days after mating using the technique of Tervit and Havik (unpublished). This technique involved injecting sheep serum into the uterine lumen near the utero-tubal junction and collecting it from a urethral catheter inserted just anterior to the uterine bifurcation. Each horn was flushed separately. The recovered eggs were examined for evidence of fertilization and stage of development and, after brief storage at 37° C, either one or two fertilized eggs transferred to each recipient. Recipients receiving two eggs had one transferred to each side of the reproductive tract. Recipients were anaesthetized with Acetylpromazine (Boots).

Post-surgical Procedures

The donor animals were injected i/m with 125 μg PGFA on March 28 to abort any unrecovered eggs and were mated by entire rams at the ensuing oestrus and for up to 3 subsequent cycles. The recipient animals were run with harnessed vasectomized rams for 2 months and oestrus recorded.

RESULTS

Synchronization of Oestrus

The mean intervals from I.V. sponge removal to onset of oestrus in donors and recipients were 2.4 ± 0.1 and 2.2 ± 0.1 days (± S.E.), respectively, and from PGFA injection to onset of oestrus in donors was 2.1 ± 0.2 days. After treatment with PMSG and PGFA the donors showed a higher incidence of oestrus than when treated with I.V. sponges (94% *v.* 77%; $P < 0.05$). There was no difference between donors and recipients in the incidence of oestrus after I.V. sponges (77% *v.* 86%).

Donor Ovarian Response

Data on the ovarian response are shown in Table 1. The F, E and OX donors produced more ovulations than G donors ($P < 0.05$). The F donors treated with 1 200 i.u. PMSG produced more ovulations than those treated with 800 i.u. ($P < 0.05$).

In 21 donors (41%) it was observed that some or all of the corpora lutea (C.L.) appeared to be undergoing premature regression. These C.L. were usually much smaller than anticipated and were pale pink in comparison with the "bright red" C.L. normally present 3 to 6 days after oestrus. In some cases C.L.

TABLE 1: DONOR OVARIAN RESPONSE

Breed	*No. Animals*	*Dose PMSG (i.u.)*	*No. (mean ± S.E.) Corpora Lutea*[1]	*Prem. Regr. C.L.*[2]	*No. Animals with Prem. Regr. C.L.*
F	10	800	6.5 ± 1.0	0.7 ± 0.6	2
	9	1 200	12.2 ± 2.0	4.7 ± 1.9	6
E	5	1 200	8.8 ± 2.1	3.6 ± 2.4	2
OX	12	1 600	7.8 ± 1.5	2.6 ± 1.0	6
	8	2 000	10.6 ± 2.5	1.8 ± 1.3	3
G	7	1 500	3.3 ± 0.6	0.3 ± 0.2	2

[1] Includes normal and prem. regr. C.L.
[2] Prematurely regressing corpora lutea.

TABLE 2: EGG RECOVERY AND FERTILIZATION

Breed	No. Animals	Type of Flush	No. of C.L. and (eggs recovered/100 C.L. observed) Ewes with Regr. C.L.[3]	Ewes with no Regr. C.L.	Total	No. of Eggs and (eggs fertilized/100 Eggs Recovered Ewes with Regr. C.L.	Ewes with no Regr. C.L.	Total
F	10	O/D[1]	42 (17) a	35 (71) b	77 (42) i	7 (57)	25 (44) k	32 (47)
	8	U[2]	37 (38) a	57 (79) b	94 (63) j	14 (29) c	45 (76) dl	59 (64)
E	3	O/D	—	26 (77)	26 (77) g	—	20 (90)	20 (90)
	2	U	18 (11)	— —	18 (11) h	2 (50)	— —	2 (50)
OX	9	O/D	48 (38) ck	43 (72) d	91 (54)	18 (83) e	31 (48) fi	49 (61) g
	7	U	32 (13) al	33 (64) b	65 (38)	4 (25)	21 (5) j	25 (8) h
G	3	O/D	8 (75)	1 (100)	9 (78)	6 (50)	1 (0)	7 (43)
	3	U	—	12 (75)	12 (75)	—	9 (100)	9 (100)
Total	45		185 (28) a	207 (73) b	392 (52)	51 (55)	152 (58)	203 (57)

[1] Oviduct
[2] Uterine
[3] Regressing corpora lutea

Within each breed and type of flush:

a *v.* b : $P < 0.001$
c *v.* d : $P < 0.01$
e *v.* f : $P < 0.05$

Within breeds, between type of flush:

g *v.* h : $P < 0.001$
i *v.* j : $P < 0.01$
k *v.* l : $P < 0.05$

of different size and apparently of different age and/or different stages of regression were found on one ovary. After this phenomenon was observed vasectomized rams were run with donors about the time of surgery and some ewes with, and some without, recorded regressed C.L. returned to oestrus within 4 to 7 days after the entire mating which preceded surgery. Blood samples were collected from 28 donors at the time of surgery and serum progesterone levels determined. The mean progesterone level (ng/ml ± S.E.) in 5 animals recorded as having all C.L. regressing was 0.7 ± 0.1, in 9 animals with both regressing and normal C.L. was 2.9 ± 0.9 and in 14 animals with no regressing C.L. was 3.9 ± 0.5 (all regressing C.L. *v.* no regressing C.L.; $P < 0.01$).

Analysis of the results in Table 1 showed that there was no difference between breeds in the number of prematurely regressing C.L. and the proportion of animals showing such C.L. The F ewes treated with 1 200 i.u. PMSG had more regressing C.L. than those receiving the lower dose of PMSG ($P < 0.05$).

Egg Recovery and Fertilization Rates

The mean total numbers of eggs recovered and fertilized eggs recovered from F, E, OX and G donors were 5.1 ± 0.8 and 2.9 ± 0.8; 4.4 ± 2.1 and 3.8 ± 2.1; 4.6 ± 1.2 and 2.0 ± 0.9; 2.7 ± 0.6 and 2.0 ± 0.8, respectively. The detailed results and analyses are shown in Table 2. The overall egg recovery rate of 52% was poor (203/392) and was influenced by the presence of regressing C.L. in the donors. In ewes where some or all of the C.L. were regressing 28 eggs per 100 C.L. (51/185) were recovered compared with 73 eggs per 100 C.L. (152/207) for ewes with normal C.L. ($P < 0.001$). The type of flush had no consistent effect on the recovery rate. The overall egg fertilization rate of 57% (116/203) was low and was not affected by the presence or absence of regressing C.L.

Recipient Conception and Lambing Data

One hundred and twelve eggs were transferred to 74 recipients. Pregnancy data were not available from 4 of these (2 died and 2, assumed non-pregnant on the basis of returns to oestrus, were slaughtered and their reproductive tracts not examined) but of the 70 remaining, 71% became pregnant. In recipients where eggs were transferred only to the uterus, 63% (20/32) of ewes became pregnant after receiving one egg and 81% (26/32)

TABLE 3: LAMBING PERFORMANCE OF AGED EXOTIC EWES

Mean No. Offspring per Ewe (± S.E.) and proportion of ewes failing to lamb (%)

Breed	*No. Ewes*	*Unoperated Ewes* 1974	*Unoperated Ewes* 1975	*No. Ewes* 1974	*1974*	*1975 Donors From Transfer*	*1975 Donors Post-op.*[1] *Progeny*	*Total*
F	19	2.5 ± 0.2 1/19 (5)	1.9 ± 0.3 3/19 (16)	15	2.3 ± 0.3 1/15 (7)	1.1 ± 0.5	1.4 ± 0.3 4/15 (27)	2.5 ± 0.5
E	—	—	—	4	1.8 ± 0.6 1/4 (25)	2.5 ± 1.6	2.8 ± 0.9 0/4 (0)	5.3 ± 2.0
OX	8	1.6 ± 0.2 0/8 (0)	1.6 ± 0.4 2/8 (25)	17	1.2 ± 0.2 4/17 (24)	1.1 ± 0.4	0.9 ± 0.2 5/17 (29)	2.0 ± 0.5
G	7	—	—	7	1.4 ± 0.3 2/7 (29)	0.9 ±0.4	0.4 ± 0.2 4/7 (57)	1.3 ± 0.4

[1] Progeny born to donors from their post-operative matings.

after receiving two eggs. The recipient pregnancy rate was not affected by using eggs recovered from donors with prematurely regressing C.L.

Lambing data were available from only 67 recipients (two of the pregnant recipients were slaughtered and the number of foetuses not recorded and the lamb from one recipient was never found). These 67 ewes received 101 eggs and produced 57 lambs (egg survival to term of 56%). This survival rate was not affected by whether one or two eggs were transferred to the uterus (61% (19/31) *v.* 55% (33/60)) and by using eggs recovered from donors with regressing C.L.

Lambing Performance of Aged Exotic Ewes Alive Spring 1975

Table 3 presents data on the 1975 lambing performance of donor ewes and the number of lambs born as the results of transfers. The lambing performance of donors in 1974, together with the performance of unoperated F and OX ewes in 1974 and 1975, is also presented.

Four F, one E and three OX donors died before the 1975 lambing. Causes were not identified but a similar proportion of unoperated ewes also died, indicating that old age was the main cause.

The results from the ewes unoperated in 1974 and 1975 show that an increase in age was accompanied by an increased proportion of barren ewes and for the F ewes, a depression in litter size. The donors showed little increase in barrenness after surgery (1974, 19%, 8/43 *v.* 30%, 13/43, 1975). However, they usually required more services to conceive after surgery than did unoperated aged F and OX and 4-tooth G and E ewes (F, 1.4 services for operated *v.* 1.1 for unoperated; E 1.5 *v.* 1.3; OX 1.9 *v.* 1.0; G 2.0 *v.* 2.3, respectively). Recovery of eggs from the Fallopian tube or the uterus did not affect either the proportion of donor animals barren or their litter size. With the exception of the E ewes, the litter size of donors after surgery was depressed relative to the donors' previous season's performance. The F, OX and G donors produced an average of approximately 1.0 extra offspring after eggs were transferred to recipients and the E produced an extra 2.5. The combination of the lambing performance results of the donors' post-surgery and of their recipients showed that the transfer exercise, when compared with the 1975 performance of unoperated animals or the previous season's performance of the donors, did increase the number of offspring born to donors of the F, E and OX breeds. However,

only in the case of the E donors did this increase reach that anticipated from the exercise (Allison, 1974).

DISCUSSION

The failure to increase effectively the number of progeny from the F, G and OX donors and to exploit the E to an even greater extent than that achieved, was due to a number of reasons.

1. The overall egg recovery rate was unsatisfactory owing to the poor recovery rate (28%) from animals having premature regressing C.L. The visual indications of C.L. regression were confirmed by the donors returning to oestrus at about the time of surgery and by the low levels of serum progesterone. Egg recovery would be expected to be lowered in animals with regressing C.L. as egg transport could be affected by the abnormal hormonal situation.

The cause of the regressing C.L. is unknown. However, donors returned to oestrus about 15 to 19 days after their I.V. sponge synchronized oestrus. This interval is approximately the length of a normal oestrous cycle and suggests that the PGFA injected 9 or 10 days after the sponge synchronized oestrus did not induce complete luteolysis of C.L. present at that time but may have depressed progesterone levels sufficiently to allow oestrus and ovulation. Subsequently the original C.L. regressed completely about 14 days after the sponge controlled oestrus and caused premature regression of the C.L. which followed the superovulation by PMSG (Inskeep *et al.*, 1963). However, other factors suggest that incomplete luteolysis may not be the cause of the prematurely regressing C.L. First, incomplete luteolysis should not result in some animals having a mixture of regressed and normal C.L. and others having C.L. at apparently differing stages of regression at the time of surgery. Also, owing to the presence of progesterone from the incompletely regressed C.L., the incidence and precision of oestrus after PGFA would not be expected to be as high as that in this study. The fertilization rate of eggs should also be affected. Thirdly, at the time of surgery no animals were observed to have regressing or recently regressed full-cycle C.L. Therefore, the short life-span of the C.L. is unlikely to be due to incomplete luteolysis of the original C.L. Instead it is probably due to the failure for some unknown reason of the normal lutectrophic mechanisms. Premature regression of C.L. has been observed after the administration of PGFA alone (B. M. Bindon, pers. comm.) and PMSG alone (M. J. Cooper, pers. comm.).

2. The egg fertilization rate was low (57%). It is not possible to ascertain whether this was due to the hormone regime used, poor ram performance, or a combination of both. Recent unpublished data suggest that the fertilization rate after a similar regime or after PGFA alone is depressed (Allison, pers. comm.). However, the performance of the "exotic" rams used must be questioned because of their poor libido, and the low and variable conception rates in the non-operated ewes mated to these rams.
3. The G ewes were not superovulated as satisfactorily as desired. The PMSG dose chosen for the breeds was based on the study of Bradford *et al.* (1971) but it is apparent that the dose chosen for the G was too low.

The proportions of recipients pregnant and eggs surviving to term were similar to those reported by other workers (*e.g.*, Moore and Shelton, 1962). Thus eggs recovered from donors with an abnormal hormonal pattern were still capable of normal development in recipients.

The advancing age of the donors probably accounts for most of the increase in barrenness and decrease in litter size between 1974 and 1975. However, the increased number of services to conception after surgery suggests that tract adhesions occurred.

In conclusion, the failure to increase effectively the number of progeny born to the "exotic" donors appears to be largely due to problems associated with the particular hormone regime chosen and to poor performance of the "exotic" rams. The hormone regime used was chosen because, in the absence of evidence to the contrary, it was anticipated to most adequately meet the requirements for precise synchronization of oestrus and satisfactory fertilization of eggs.

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REFERENCES

Allison, A. J., 1974: *Proc. N.Z. Soc. Anim. Prod., 34*: 167.
Bradford, G. E.; Quirke, J. F.; Hart, R., 1971: *Anim. Prod., 13*: 627.
Hunter, G. L.; Adams, C. E.; Rowson, L. E. A., 1955: *J. agric. Sci., Camb., 46*: 143.
Inskeep, E. K.; Oloufa, M. M.; Pope, A. L.; Casida, L. E., 1963: *J. Anim. Sci., 22*: 159.
Moore, N. W.; Shelton, J. N., 1962: *Aust. J. agric. Res., 13*: 718.
Rowson, L. E. A.; Adams, C. E., 1957: *Vet. Rec., 69*: 849.