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Development of an effective goat embryo transfer regime

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ABSTRACT

Trials between 1979 and 1984 with Angora or Saanen donors showed that synchronisation of oestrus with progesterone injections delayed oestrus compared with progestagen sponges and that this delay was associated with fewer follicles and increased egg recovery and fertilisation rates. Highest ovulation rates were achieved when FSH was injected instead of PMSG and when donors were Saanens rather than Angoras. Surgical egg recovery rates decreased in donors with prematurely regressing ovulations. The response of recipients (feral or Angora) to sponge treatment varied markedly between years. Recipient pregnancy rate and embryo survival both increased when recipients were surgically twinned rather than singled.

The various trials have enabled the development of an effective transfer regime. This involves synchronising/superoovulating donors with progesterone/FSH and twinning or tripletting of recipients. Under these conditions Angora and Saanen donors average respectively, 14.0 and 24.5 ovulations, 10.8 and 18.6 transferable embryos and 7.5 and 11.8 kids born to recipients.

Keywords Goats; breeds; embryo transfer; oestrus; superovulation; fertilisation; embryo survival

INTRODUCTION

The current interest in Angora (A), Cashmere and, to a more limited extent, Saanen (S) goats has led to a demand for the rapid multiplication of the limited numbers of animals. This can best be met through the technique of embryo transfer.

This paper summarises the factors found to affect the success of embryo transfer trials at Wakelins Farm between 1979 and 1984. It outlines the evolution of a regime which effectively superovulates both A and S donors and leads to very rapid rates of reproduction.

MATERIALS AND METHODS

Trials utilising a total of 62 A and 17 S donors were conducted between 1979 and 1984. Treatment commenced during March each year and at this time all donors were cycling and varied in age from 1.5 to 8 years.

Synchronisation of Oestrus and Superovulation

The 1979 donors (10 A) were synchronised with intravaginal sponges impregnated with 60 mg medroxyprogesterone acetate (MAP, Upjohn, USA) inserted for 16.5 to 17.5 days. In all other years synchrony was achieved by daily intramuscular (i/m) injections with 12 mg progesterone (Sigma, USA) in peanut oil for 17 days. The 1979 donors were superovulated by an i/m

injection of pregnant mares' serum gonadotrophin (PMSG; Paines and Byrnes, UK) administered 1.5 days before sponge removal and the 1980 (10 A) and 1981 (15 A) donors with PMSG injected at the time of the sixteenth progesterone injection. Half the 1982 donors (8 A) were superovulated as in 1980-81 while the remaining 1982 donors (8 A) and all the 1983 (6 A, 7 S) and 1984 (5 A, 10S) donors received 4 mg follicle stimulating hormone (FSH, Burns-Biotec, USA) i/m at the time of the sixteenth progesterone. This was followed at approximately half daily intervals by consecutive doses of 3, 3, 3, 2, 2, 2, 2 mg FSH so that a total of 21 mg was injected over 4 days.

Immediately after sponge removal or the last progesterone injection, each donor was placed with her allotted harnesses, entire buck and observed at 6 to 8 hourly intervals for oestrous activity and mating marks.

Each year, recipient feral and A does were synchronised with MAP sponges inserted for 16.5 to 19.5 days. Sponge removal was timed so that about 7 to 10 recipients were expected to show oestrus about the time of each donor. At sponge removal recipients were placed with harnesses, vasectomised bucks and observed twice daily for mating marks. Marked does were removed from the flock at each mating examination.

Surgery and Egg Handling Procedures

Surgery was performed between 4 and 6½ days after mating. The treatment of donors and recipients before and after surgery was as described previously (Tervit *et al.*, 1983, 1985). Eggs were flushed from each donor's uterus in 40 ml (20 ml per uterine horn) warm (37°C) Dulbecco's phosphate-buffered saline (Oxoid, UK) enriched with 10% heat-treated vasectomised buck serum. The flushings were examined, any eggs located and transferable embryos stored at 37°C until transfer. The recipients were subjected to normal surgical embryo transfer procedures. They received either one or two embryos into the uterine horn ipsilateral to a functional corpus luteum (CL), one embryo transferred to each uterine horn or, two embryos to the ipsilateral and one to the contralateral horn.

RESULTS AND DISCUSSION

The major factors affecting the efficacy of the embryo transfer regime were:

Donor Synchronisation Drug

Donors treated with sponges in 1979 showed oestrus earlier than those treated with progesterone injections in 1980-81 ($P < 0.05$, Table 1). This earlier onset was disadvantageous as it was associated with more large follicles ($P < 0.01$), decreased egg recovery ($P < 0.001$) and fertilisation rates ($P < 0.01$) and a lowered incidence of transferable embryos ($P < 0.01$). This poor performance of donors after sponge treatment could be due to hormone imbalance effects because of the relatively short interval between PMSG injection and onset of oestrus. The goat has an oestrous cycle of similar length to the cow and therefore, like the cow, is likely to give the best response to superovulation when the interval from PMSG to oestrus is at least 3 days (Hafez *et al.*, 1965). This is achieved after progesterone injections and so this injection regime is the one currently recommended for donor synchronisation.

Donor Superovulation Drug

The A donors superovulated with FSH in 1982

produced more ovulations ($P < 0.05$), eggs ($P < 0.1$), embryos ($P < 0.05$) and more transferable embryos ($P < 0.05$) than those treated with PMSG (Table 2). This superiority of FSH has been recorded by others for goats and sheep (Armstrong and Evans, 1983) and cattle (Elsden *et al.*, 1978) and is thought to be due to the considerably more rapid clearance of FSH than of PMSG from the circulation resulting in low levels of circulating exogenous gonadotrophins at oestrus and ovulation. Progesterone and FSH injections are now used routinely by most commercial goat embryo transfer groups to synchronise/superovulate donors. The groups accept the relatively tedious procedure of multiple injections because of the very high ovulation rates and number of transferable embryos achieved.

Donor Breed

On average, S produced more ovulations ($P < 0.001$), eggs ($P < 0.001$), embryos ($P < 0.001$) and more transferable embryos ($P < 0.001$) than Angoras (Table 3). Donor breeds with high fecundity usually superovulate more readily than low fecundity breeds (Tervit *et al.*, 1976) and superiority of the S was expected. In the present study there was however a strong breed x year interaction ($P < 0.01$). This was partly due to poorer than expected performance of the 1984 S donors (particularly their low embryo recovery rate) and to very poor performance of the 1983 A donors. The transfers in 1983 were conducted during severe drought conditions and the A, unlike the S which were newly purchased and were being fed supplementary feed, were not supplemented and were losing weight before and during the transfer programme. This uncharacteristically poor performance of A donors highlights the importance of donor nutrition to a successful transfer programme.

TABLE 2 Effect of superovulation drug on ovulation and embryo response (mean numbers).

Hormone	Ovulations	Eggs recovered	Embryos recovered	Transferable embryos
PMSG	9.1	7.8	6.0	5.1
FSH	15.1	13.0	12.1	11.0

TABLE 1 Distribution of oestrus after synchronisation.

Hormone treatment	No. animals showing oestrus by various intervals (days) after treatment										Mean interval (days)
	1	1½	2	2½	3	3½	4	4½	5	5½	
Sponge/PMSG	6	—	—	—	—	1	1	—	—	1	2.1
Progesterone/PMSG	—	—	1	3	6	5	5	—	3	2	3.6

Prematurely Regressing Ovariations

Thirteen percent of the donors treated over the 6 years (year range, 0 to 27%) had CL undergoing premature regression. The phenomenon was not confined to any hormone treatment or breed and affected all CL present. It was characterised by CLs of small size and pale colour and donors which had either returned to oestrus or were shortly to do so. Egg recovery rates in donors with prematurely regressing CL were much lower than in donors with normal CL (49% v 87%, respectively, $P < 0.001$). The phenomenon of luteal failure and early return to oestrus in superovulated goats has been reported by a number of authors (eg. Armstrong *et al.*, 1982). It leads to abnormal egg transport and hence poor egg recovery rates. Reasons for the failure are unknown but in some studies the majority of animals are affected (Armstrong *et al.*, 1982). Good egg recovery rates can be achieved in luteal failure animals by flushing the eggs from the tract early after onset of oestrus. The early flushes are oviduct flushes and, because these cause more reproductive tract adhesions than uterine flushes (Tervit and Havik, 1976), we chose to continue to conduct uterine flushes. Interestingly, embryos recovered from luteal failure donors have satisfactory survival rates in recipients.

Sires Used

In some years the sires used affected the percentage of recovered eggs fertilised. The overall fertilisation rate was 91% but in 1979-1981 low fertilisation rates were associated with mating to old sires (6 to 10 years old, 81% eggs fertilised; 2 to 3 years old, 91%; $P < 0.05$). In 1982, the sire again affected egg fertilisation ($P < 0.001$),

the affect being mainly attributable to one young sire giving a low rate (65%). It was presumed that sperm quality, although unexamined, was a major factor contributing to these sire effects.

Recipient Oestrous Response

Failure of recipients to show a synchronised oestrus after sponge removal has a major affect on the efficacy of any embryo transfer programme. The response of recipients to the MAP sponges is shown for the various years in Table 4. There was a significant year effect on the proportion of recipients showing an oestrus after device removal ($P < 0.001$) and on the distribution ($P < 0.001$) and mean interval ($P < 0.001$) to that oestrus. There was no difference between years in the highest percentage of does showing a synchronised oestrus over a 24 or 48 h period. In 1979 alone recipients were treated with sponges for 19.5 days. These animals showed oestrus later than animals treated for shorter periods ($P < 0.05$). Other reasons for the variation in recipient response are unknown. An attempt is currently being made to improve recipient synchrony and incidence of oestrus by injecting a low dose of PMSG at sponge removal. Preliminary results are encouraging in terms of oestrous response but limited recipient data suggest that embryo survival may be lowered in the PMSG treated animals. In some veterinary groups recipients are being synchronised with a new device, the controlled internal drug release device (CIDR). It appears to be giving satisfactory results with recipients showing oestrus about a day earlier than similar animals treated with sponges (1.5 v 2.4 days, respectively).

TABLE 3 Effect of donor breed on ovulation and embryo response (mean numbers).

Breed	Year	Ovulations	Eggs recovered	Embryos recovered	Transferable embryos
Angora	1983	5.3	5.2	5.2	5.0
	1984	12.2	10.4	10.4	10.4
Saanen	1983	29.3	27.1	27.1	25.3
	1984	21.1	15.5	14.1	14.0

TABLE 4 Distribution of oestrus after recipient synchronisation.

Year	No. animals			No. animals showing oestrus by various intervals (days) after treatment											Mean interval (days)
	Treated	Showing oestrus*	(%)	1	1½	2	2½	3	3½	4	4½	5	5½	6	
1979	61	57	(93)	1	9	10	17	4	10	2	3	—	1	—	2.6
1980	86	80	(93)	1	19	16	25	8	6	1	2	—	—	2	2.4
1981	127	107	(84)	2	4	25	13	18	18	17	6	2	2	—	3.0
1982	182	136	(75)	—	4	7	8	47	20	28	9	4	4	5	3.5
1983	161	118	(73)	—	1	23	24	28	23	9	3	3	1	3	3.1
1984	155	114	(74)	—	5	10	21	24	25	7	8	4	8	2	3.4

* Showing oestrus within 7 days of device removal

Recipient/embryo Performance

The kidding performance of recipients and the survival of embryos has a major effect on the efficacy of any transfer regime. Interestingly, in the goat these parameters were surprisingly little affected by factors examined.

Kidding rate in 1979-1981 was lower when the recipient was singled than when twinned (58% v 80%, $P < 0.01$). Embryo survival also tended to be higher in twinned animals (58% v 70%, respectively, $P < 0.1$). This superiority of twinning is in contrast to the sheep where, when more than one embryo is transferred, pregnancy rates are unchanged but embryo survival decreases (Moore, 1968). The goat uterus appears more able to support multiple pregnancies and embryo quality, rather than uterine environment, is likely to have the major effect on survival. The substantial uterine capacity of the goat is supported by our observation that an increase from 2 to 3 embryos transferred per recipient had no effect on kidding rate or embryo survival. In 1983, both kidding rate and embryo survival were affected by the breed of embryo transferred with A-derived embryos performing poorly. This was due to a problem with embryo quality since recipients from the same recipient "pool" gave satisfactory results with S embryos. Each year an attempt was made to closely synchronise onset of donor and recipient oestrus ($\pm \frac{1}{2}$ day). However, in no year was kidding rate or embryo survival affected by the closeness of synchronisation of the donor and recipient oestrus (recipients showed oestrus from 1 day earlier to 2 days later than the donors). This is unlike the cow and sheep (Lawson *et al.*, 1975; Moore and Shelton, 1964) where close synchrony of donor and recipient oestrus is necessary for satisfactory pregnancy rates. The observation by Armstrong *et al.* (1982) that embryo survival in the goat is positively correlated with the number of ovulations on the recipient ovaries was observed in only one year (1982). In that year an increase in ovulation rate from 1 to 2 was associated with an increase in survival from 56 to 73% ($P = 0.05$). Finally, the 1984 study showed that in unilaterally ovulating recipients, embryo survival was not affected by whether recipients were twinned by transferring one embryo to each uterine horn or by transferring both embryos to the uterine horn ipsilateral to the ovulation(s).

In conclusion, the various trials have enabled the development of an effective and widely used transfer regime. This involves synchronising/superovulating well fed A, Cashmere or S donors with progesterone/FSH and surgically twinning (by transferring 2 embryos to the uterine horn ipsilateral to functional ovulation(s) or tripletting recipients (by transferring 2 embryos to the ipsilateral horn and 1 to the contralateral horn). Under these conditions A and S donors average respectively, 14.0 and 24.5 ovulations, 10.8 and 18.6 transferable embryos and 7.5 and 11.8 kids born to

recipients. The effectiveness of the technique will be improved further when more effective recipient oestrous synchronisation techniques are perfected and through the development of embryo recovery and transfer techniques which allow the donor and recipient reproductive tracts to remain in the abdominal cavity during manipulation. The latter techniques are currently being developed and involve examination of the reproductive tract through an endoscope and flushing embryos out of or transferring embryos into the tract through appropriate catheters and pipettes. The techniques subject the uterus to minimal manipulation and, once perfected, should allow donors and recipients to undergo more transfers than is possible following current full surgical procedures.

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