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EGG TRANSPLANTATION STUDIES IN ROMNEY EWES

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SUMMARY

The application of egg transfer techniques in studies of reproductive performance of sheep was examined in relation to two trials with Romney ewes. In Trial 1, fertility was compared after egg transfers within and between sheep and in mated ewes after laparotomy without egg transfer. In Trial 2, egg transfers between sheep were made and survival of embryos examined.

Overall, the conception rates following egg transfers were about 50%, although less than in mated ewes examined at laparotomy. Transfers of eggs between ewes rather than within ewes appeared to have advantages as a technique in certain studies on reproductive performance.

THE REMOVAL of gametes or zygotes from the reproductive system of one female and their transfer and subsequent development in other females has often been suggested as a method for propagating desirable genotypes. Of the farm species, the sheep has been extensively studied to determine the factors critical to the successful application of such techniques. In considering methods for transplantation of gametes, it should be realized that successful transplantation might be undertaken at various stages of the reproductive process. While there is the possibility of transplanting ova recovered directly from follicles or even transplanting the ovaries or portions of ovaries into recipient females *prior to* ovulation, most work has been concerned with gamete transfer *after* ovulation. Thus, transfer techniques successfully used in the sheep have included (a) recovery of ova immediately after ovulation in the donor and their fertilization attempted in the recipient ewe (Woody and Ulberg, 1963), (b) transfers of fertilized but uncleaved ova, (c) transfers of fertilized ova at various cell stages (2-cell to 16-cell morulae) (Averill and Rowson, 1958), and (d) transfers of blastocysts or embryos up to approximately 13 days after onset of oestrus in the recipient (Moor and Rowson, 1966).

The most successful results of gamete transfer, measured by conception rate in ewes, have probably been achieved with fertilized eggs of the 8-cell stage or somewhat older, transferred to the uteri of synchronized ewes.

In several studies, conception rates of the order of 70% were achieved (*e.g.*, Moore and Shelton, 1964). Application of egg-transfer technique as described is also satisfactory for two practical considerations. First, 8-cell ova may be recovered *in vivo* 2½ to 3 days after the onset of oestrus; at this time they are usually located within the Fallopian tubes and, upon flushing with homologous serum or other fluid, a high proportion of eggs can be found. Where recoveries of older eggs are attempted, then lowered percentages are usually found and this is probably owing to the greater volume of uterine tissue that needs to be flushed with fluid and searched. The second practical reason for transferring 8-cell eggs is that placement of eggs directly into the uterus is also simpler than insertion of "young" eggs into the ampulla of the Fallopian tube.

Notwithstanding the successful demonstration of egg transfer, the application of this technique in commercial animal production has been little attempted and for very good reasons. As far as breed improvement is concerned, the method holds little promise relative to the difficulty of selecting those animals of superior merit, and a further reason against attempts to select and use widely the desirable females is that, for the important productive characteristics like fitness, growth and reproductive performance, the productivity of the crossbred is generally greater than for the parent breeds, and of course crossbreds are rapidly propagated through relatively few sires. Nevertheless, egg transfer may yet have some role in a sheep industry, as a means for initially propagating a recently introduced breed or for increasing the number of animals within a breed which show some genetic characteristic of important commercial value.

In this respect, it is worthy of mention that egg transfer to increase the number of Drysdale ewes is being applied in New Zealand. Drysdale wool, because of the properties imparted through the N-gene (especially in the homozygous condition) and the supply-contract entered into between the producer and the manufacturer, commands a premium in price above that for the usual Romney wool. Egg transfer of Drysdale eggs into Romney ewes to act as "incubators" is currently under way.

While commercial uses of egg transfer have been of little significance in farm animals, there is no doubt that these techniques as an "experimental tool" have been of considerable value in studies of animal physiology and production. It is of interest to recall that the first lambs born as a result of egg transfer in New Zealand arose from

experimental studies concerning failures in fertilization of ova in sheep mated at various stages of the breeding season. In that work cited by Laffey and Hart (1959) and D. S. Hart (pers. comm.) at Lincoln College, Corriedale lambs were born to, or embryos present in, Southdown ewes after transfers of fertilized 1-cell ova as well as eggs from 2- and up to 16-cell stages at time of transfer.

In work at Massey University with Romney ewes, egg transfer has been used as a means of *avoiding* fertilization failure in the recipient and it has allowed variable numbers of eggs to be transplanted. The ewes which received the eggs have differed in characteristics such as rearing rank, liveweight or face cover, and the effect of these characteristics on survival of fertilized eggs have been noted. The results from two trials have been chosen to illustrate some problems associated with application of egg-transfer techniques in these types of studies.

EXPERIMENTAL

TRIAL 1 (1963)

Ninety aged Romney ewes (3 groups of 30 sheep) were run with vasectomized rams from mid-January and observation made for first, second, and third oestrus of the breeding season. Ewes in Groups 1 and 2 were mated to fertile rams at third oestrus. Laparotomies were conducted after both first and third oestrus and the numbers of fresh corpora lutea recorded as evidence of number of ova shed. At third oestrus, recovery of ova was attempted in ewes of Group 2 and if all eggs were found and were fertilized they were transferred back into the same sheep. Group 3 animals at third oestrus received fertilized eggs from synchronized donors; the number of eggs transferred (1 or 2) was the same as the number of recent corpora lutea recorded in the ovaries of the recipients. Half of the pregnant sheep in each group were slaughtered 20 to 23 days after oestrus; the remainder were designated to go to term.

TRIAL 2 (1964-65)

Egg transfers were attempted between donor ewes and some 170 potential recipient ewes. In order to examine certain factors which might influence reproductive performance, and as well to reduce labour involved in detection of oestrus and mating of ewes and to reduce the period during which the surgery was undertaken, the

oestrous cycles of the sheep were regulated by injections of progesterone. These injections commenced over a 12-day period from about February 18 in both years. Each day a constant ratio (1964, 5:10 — 1965, 9:7) of donor to recipient ewes was injected. Vasectomized rams were with all ewes from just before progesterone treatment until after oestrus had occurred or ovulation without oestrus was assumed to have occurred. Entire rams were then run with the donors to allow fertile services at the next oestrus, but the recipients remained with "teaser" rams.

Induction of superovulation in donors was attempted by subcutaneous injection of 500 to 1,500 i.u. pregnant mares' serum (PMS) 11, 12 or 13 days after first oestrus. For donors which were not in oestrus after progesterone treatment, an estimate of the time of ovulation was made and PMS administered 12 days later.

Fertilized ova (1, 2 or 4) were transferred to recipient ewes synchronized with respect to onset of oestrus in the donors.

Sheep which did not return to oestrus within 19 to 22 days after oestrus (some within 35 days in 1964) were slaughtered and observations made on the embryos.

RESULTS AND DISCUSSION

TRIAL 1

Table 1 shows the number of ewes examined at third oestrus and data for ewes which were pregnant. Four ewes were not examined after third oestrus owing to death (one ewe) or incorrect recording of oestrus (three ewes).

The conception rates for ewes in Groups 1, 2 and 3 were 72%, 49% and 64%, respectively. The incidence of pregnancy in the ewes of Group 1 is probably similar to or only slightly less than that usually found with ewes which are mated at a single oestrous period. The stress of laparotomy (in this experiment conducted under general anaesthesia) might be expected to reduce conception rate (Lamond,

TABLE 1: NUMBERS OF LAPAROTOMIES AND EGG TRANSFERS CONDUCTED IN EWES AT THIRD OESTRUS AND OF EMBRYOS DEVELOPING OR LAMBS BORN

Group	No. of Laparotomies	No. of Transfers	No. of Ewes	Pregnant Ewes		
				Killed No. of Embryos	at Term No. of Ewes	No. of Lambs
1	29	—	12	16	9	11
2	28	21	5	8	5	7
3	29	14	4	6	5	8

1963) although from these results it may be inferred that the effect of laparotomy need not be excessive. Where manipulation of the reproductive tract and transfer of eggs are attempted (Groups 2 and 3) then conception rate might be expected to be lower than in ewes not subjected to laparotomy or for sheep such as in Group 1.

"Between-sheep" egg transfers yielded a satisfactory conception rate (9 of 14 ewes pregnant). However, few transfers of the ewes available were in fact conducted and this result was owing to the experimental limitations imposed; transfers were attempted only at third oestrus between "donors" and "recipients" synchronized with respect to onset of oestrus. To increase the number of transfers made, a larger number of potential donors were required than the 30 that were available.

"Within-sheep" egg transfers (Group 2) yielded the lowest conception rate compared with the other techniques employed to study reproductive function. The removal of eggs from a reproductive tract, examining them for evidence of fertilization or other changes and then, after replacement back into the genital tract, examining their survival at intervals is an attractive technique for studies of reproduction. However, such a technique needs to be considered in relation to the reduction in conception rate and increased loss of potential offspring. There is little evidence as to the reasons for the decrease in conception rate, especially since both the age of the egg and state of the reproductive tract should be synchronized. However, it may be suggested that the survival of ova *in vivo* is unlikely to be aided by the flushing of fluid through the uterus and Fallopian tubes to recover the eggs initially. In addition, with ova of 4-cell stage and less and which should be returned to the Fallopian tubes (Averill and Rowson, 1958), the excess flushing fluid which remains may facilitate easy loss of ova from the ampulla region after transfer.

The usefulness of "within-sheep" transfers is also reduced owing to wastage of potential recipients (7 of 28 ewes in Group 2). This wastage in ewes arises because (a) not all ova are recovered from the genital tract, hence further results with such ewes may be difficult to evaluate, and (b) some ova are not fertilized.

The number of embryos present at 20 to 23 days of pregnancy for ewes in all groups gave a good indication of the number of lambs born in similarly treated ewes allowed to proceed to term. This finding is in agreement with other studies (e.g., Moore *et al.*, 1960).

TRIAL 2

Detailed results of this work will be published elsewhere, but, in summary, 36, 68 and 36 recipient ewes were transplanted with 1, 2 or 4 fertilized eggs, respectively, and the conception rates in these groups of ewes were similar (50%).

Transfer of eggs between ewes is feasible with reasonable numbers of sheep examined at a limited period of time and the introduction of variable numbers of fertilized eggs to "load" the reproductive tract more than normal would seem to offer a practical method of studying differences in reproductive performance. Synchronization of oestrous cycles in donors and in recipients by injection of progesterone or some other progestagen treatment, and the programming of the number of transfers to be attempted on particular days, facilitates the conduct of such experiments.

In the present study, the injection of progesterone ceased in these ewes about the start of the normal breeding season, but only 65% of treated ewes were in oestrus within a few days of progesterone withdrawal (Cumming and McDonald, 1967). However, as nearly all ewes ovulated, subsequent treatment of donors to cause super-ovulation and oestrus was successful, while most potential recipients came into oestrus near the predicted time prior to the anticipated transfer. Appreciable numbers of donors are required for between-sheep transfers, even where about normal numbers of eggs per recipient are required. Considerable variation in the number of eggs per ewe treated with PMS and available for transfer may occur. With the Romney ewes in this experiment, the ratio of one "donor" to one or two "recipients" was satisfactory. Nevertheless, the super-ovulation response to PMS is not particularly precise and wastage of eggs and wastage of potential recipients are difficult to avoid; the former loss is preferred.

The conception rates in the groups of ewes transplanted with 1, 2 or 4 ova were similar (50%). For several reasons, certain transfers were not conducted at optimum times and not all eggs were placed in the uterus; however, such transfers were distributed at random between the three groups. There were, however, differences in the percentages of eggs surviving as embryos and seen at autopsy. For ewes which received 1, 2 or 4 ova each, 53%, 77% and 133% embryos, respectively (calculated as $\text{No. of live embryos} \div \text{No. of ewes "transferred"} \times 100$) were found.

Because the number of embryos present at this stage of pregnancy gives a good indication of number of lambs likely to be carried to term, the practical implication of this result is that the number of ova released into the genital tract and fertilized is a critical determinant of the number of lambs born. Factors in management of sheep which increase the numbers of ova shed at mating or methods for the selection for breeding purposes of those sheep with a propensity for high ovulation rate are worthy of investigation in Romney ewes.

Information on ovulation rate in recipient ewes is provided in the course of egg transfer experiments at laparotomy. In Trial 1, the data were obtained for ewes at third oestrus and this occurred naturally during the breeding season. In Trial 2, the ovulation rate of ewes may have been influenced by the progesterone induction of ovulation, although the recorded corpora lutea were the result of ovulations about a cycle-length after the end of progesterone-treatment. The mean number of corpora lutea per ewe was 1.4 and this was about the same as for similar ewes experiencing oestrus naturally and in the same environment. The data from both experiments reveal that an appreciable percentage of 5½- and 6½-year-old Romney ewes ovulate only one egg at oestrus.

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