TRANSPORT OF SHEEP OVA IN RABBITS

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SUMMARY

The feasibility of using rabbits for the transport of sheep ova from overseas to New Zealand was examined. In one trial, Merino ova from Australia were imported in rabbits and transferred to Romney ewes and some pregnancies resulted. The results of preliminary studies to examine aspects of the egg-transfer technique which may limit its usefulness under New Zealand conditions were presented.

THE New Zealand Department of Agriculture is setting up a maximum security quarantine station on Somes Island. This will make possible for the first time for many years the introduction of sheep into this country from countries other than Australia. Most of the breeds that are being considered for importation are from Europe and the purchase and transport costs of bringing these animals here will be very high. In the present paper, the technique of transport of sheep ova inside rabbits is reviewed and discussed as a possible way of reducing these costs of animal importation.

Consideration is also given to the case for the introduction of new breeds of sheep to New Zealand and the relationship of the egg-transfer technique to that objective.

TRANSFERS OF SHEEP OVA TO RABBITS

Averill *et al.* (1955) transferred 18 2- to 12-cell sheep ova to the Fallopian tubes of pseudo-pregnant rabbits and after 4 to 5 days, nine eggs were recovered and found to have continued to develop normally. Three of the eggs were transferred to non-pregnant recipient ewes and two normal embryos were later recovered. The possible use of rabbits as an incubator for the long-distance transport of sheep eggs was first mentioned by these workers. In 1960, sheep ova were sent from England to South Africa (Adams *et al.*, 1961). Forty-five ova were transferred to three rabbits, and in South Africa 23 ova were recovered and transferred to 10 ewes; six pregnancies resulted. This experiment remains the main published work in this field. It is reasonable to include here the comment by one of the South African participants in this experiment that their group had had no

previous experience in handling the reproductive tract of rabbits and that the results may have been affected by this.

To study development of sheep eggs in rabbits, Adams *et al.* (1967) transferred 8- to 32-cell sheep ova to oestrous rabbits. Four to nine days later, 19 of 20 eggs were recovered, and only one had failed to develop. At recovery, 3 of the original ova were classified as "somewhat degenerate", 15 were advanced blastocysts of which 5 had shed their zona pellucida. Therefore early development of sheep ova and shedding of the zona are independent of species, organ and endocrine status. None of the above embryos was transferred back to sheep.

At Ruakura, during the 1968 breeding season (Welch, unpubl.), 61 eggs were transferred to the Fallopian tubes of 12 pseudo-pregnant rabbits for five days; 50 eggs were recovered from the rabbits and, of these, 23 eggs probably continued to develop while in the rabbits. Sixteen eggs were subsequently transferred to sheep and 6 lambs were born.

In a further trial (November, 1968) ova were removed from Merino ewes in Sydney and transferred into Romney ewes at Ruakura (Welch and Humphrey, unpubl.). Fourteen ova were transported *in vitro* in sheep serum at 10° C, and 8 were transferred to four recipient ewes; none of these ewes was pregnant two months later. At the same time 45 ova were transferred to three rabbits in Sydney and 36 eggs were later recovered at Ruakura. Most of the discrepancy in number of eggs was due to the complete loss of five eggs from one Fallopian tube. Twenty of the ova were transferred to ten recipient ewes, and at laparotomy two months later two of four ewes were pregnant. Further importations of sheep ova from Sydney are planned for the 1969 breeding season.

FACTORS AFFECTING EGG-TRANSFER TECHNIQUES

Several aspects of the egg-transfer technique may affect it usefulness as a means for importing sheep to New Zealand. These are discussed as follows:

Superovulation of Donor Ewes

Cumming and McDonald (1967) showed that increasing amounts (500-1,500 i.u.) of pregnant mares' serum (PMS) gave increasing ovulation rates in Romney ewes, but at the higher doses (resulting in about 9 ovulations), there was a wide range of responses, and that, for ewes ovulating more than ten ova, fertilization failure was high. Moore

and Shelton (1964) showed that horse pituitary powder gave better superovulation of Merino sheep than did PMS, largely by decreasing the numbers of unruptured follicles. Tests of pituitary powders and extracts for their ability to induce superovulation in Romney sheep (Welch, 1969) have shown that sheep pituitary powder is quite unsuitable as large numbers of cystic follicles formed and frequently ovulation occurred soon after the injections were commenced; there was also failure of fertilization. This effect with high doses of PMS was also noted by Cumming (1965). Partially purified horse pituitary follicle stimulating hormone (FSH) (fraction CM-1; Hartree *et al.*, 1968) was very satisfactory in Romney eyes and gave high ovulation rates, low numbers of cystic follicles, and high fertilization rates. If human chorionic gonadotrophin (HCG) is added to the horse FSH, then cystic follicles occurred, suggesting that it is the luteinizing hormone activity of gonadotrophin extracts that causes the unovulated follicles and unfertilized eggs.

Controlled Ovulation in Recipients

It is important that the time of ovulation of recipient ewes be closely controlled, as the percentage success of ovum transfers (as determined by the viability of embryos) falls if the stage of development of the embryo and the uterus are not synchronized (Moor and Rowson, 1966). Using a modification of the sequence described by Braden *et al.* (1960), of a progestogen-impregnated sponge inserted into the ewe for 10 days, 1,000 i.u. PMS at the time of sponge withdrawal followed 24 hr later by 500 i.u. HCG (iv) complete success was achieved in inducing ovulation in 66 ewes in early February (Welch and Bellve, unpubl.). Similar treatments of 37 ewes in October resulted in 22 ewes ovulating only. As sheep ova from Europe would probably be recovered and sent at the time sheep are anoestrus in New Zealand, it is important that the technique for induction of controlled ovulation at this time of year be improved.

Numbers of Ova to Transfer to Each Recipient Ewe

If ova are sent to this country, it will be most important that the maximum number survive. This will be more important than the proportion of recipients that become pregnant. Cumming (1965) found that the transfer of one, two or four ova per ewe did not increase the proportion of

ewes that became pregnant. However, the likelihood of survival of any one ovum was better if it was transferred as a single ovum rather than one of a multiple egg transfer. Similar experiments, although with smaller numbers of ewes, have given very similar results at Ruakura. In both these studies, the percentage success of the transfers was about 50%, which is less than the results recorded in some other trials (*e.g.*, 75%; Moore, 1968).

DISCUSSION

A large-scale experiment to test thoroughly the use of the technique of egg transfer for introduction of sheep to a country has yet to be done. However, the results described here have encouraged the planning of further work at Ruakura for the 1969 breeding season. Considering the available evidence, it would seem that egg-transfer may have a role if techniques can be developed that reliably produce an average of 10 fertilized eggs per donor ewe, together with at least 20% of these eggs surviving transfer to rabbits and then back to sheep. Thus it might be expected that, as a result of transfer, each donor ewe on average should yield at least two lambs. On the assumption that this is possible, some discussion should be made concerning the possibilities of the use of the technique in New Zealand with particular reference to a recent article by Quinlivan (1968). That author criticized both the idea of introducing new breeds of sheep to New Zealand and also of the use of the technique of egg transplantation.

Quinlivan has asserted that the technique of egg transfer in rabbits has failed to be of practical application in the United Kingdom, Russia, South Africa or Australia. While there is no disagreement with that assessment of the use of egg transfer so far, it is also clear that earlier occasions of transfers between countries have been to *demonstrate the technique, not to introduce new genetic material.* Factors which have led the United Kingdom, Russian and South African workers not to use the technique in a practical manner as now envisaged could include some or all of the following: Much lower transport costs of desired imports of stock compared with costs from Europe to New Zealand; quarantine regulations for sheep less stringent than in New Zealand; lack of suitably experienced personnel to handle the technique; and also the fact that these countries began purchasing exotic breeds, notably the Finnish Landrace, well before New Zealand showed interest and before the price of breeding stock of particular

breeds showed its marked increase over the last few years. In the case of Australia, there is a complete embargo on the importation of farm livestock including ova and semen. These factors are different in New Zealand.

Egg transfer in rabbits between countries, as with most techniques, would not replace existing methods, but would extend the range of objectives. For example, if it is intended to test Finnish Landrace or Oldenburgh sheep or their crosses at a Government research station in New Zealand, then donor ewes could probably be obtained in Britain and their ova transported to New Zealand in rabbits. This would make sheep available that are not on the market in Britain, and would eliminate the alternative of purchase on the continent of Europe and bringing them through the much stricter quarantine regulations that would then apply. Considering another example, if it is desired to import sheep from New South Wales, Australia, it is necessary that quarantine precautions be satisfied in New Zealand to ensure that the sheep are free of nodule worm, *Oesophagostomum columbianum*. Utilizing the egg-transfer technique, fertilized ova could be (and in fact, have been) brought directly into New Zealand from any part of Australia without any quarantine restrictions.

The above examples are advanced to show that the use in New Zealand of the technique of egg transplantation might make available the offspring of sheep that could not normally be obtained, and also, in some instances, lower or eliminate quarantine restrictions. These advantages are additional to the obvious benefits of reducing costs in purchase and transport. These comments also apply to exports from New Zealand. For example, in a recent request assistance has been sought in sending ova from New Zealand Romney sheep to Kenya.

Quinlivan (1968) also doubts whether exotic sheep breeds if introduced to New Zealand would have much impact on our national flock of about 50 million. While there would be agreement that the proposed facilities for sheep quarantine on Somes Island are small, they should nevertheless be adequate to introduce enough representatives of any breed intended for testing under New Zealand conditions. If there are breeds which show definite advantages, it is hoped that larger facilities would be available for commercial introduction.

In the preliminary stages of "breeding up" such desirable sheep, egg transfer from sheep to sheep would also seem to have a role. With efficient superovulation techniques, one donor ewe, if subjected to 3 laparotomies for egg

recovery in 6 cycles in one season, should be able to leave 25 lambs per season. The degree of success in sheep-to-sheep transfers is very satisfactory and approximately 75% of transferred eggs have survived under optimum conditions (Moore, 1968). Moreover, one may also speculate that there is the possibility of shortening the generation interval by superovulation and recovery of eggs from lambs for transplantation into ewes of existing breeds (Land and McGovern, 1968). Furthermore, through artificial insemination, a few sires of the desired breeds could have a marked influence in increasing the number of cross-bred progeny out of the ewes of the breeds already present in New Zealand. Taken together, it is believed that techniques exist for multiplying very quickly a few dozen sheep to a few thousand. Even so, it is going to take a long time for this number to make much impression on the national flock. This does not mean that we should not begin — only that it is a pity exotic breeds were not introduced years ago, and that the sooner these are introduced the better.

Finally, Quinlivan has also doubted whether exotic breeds have any part to play in New Zealand and whether we can assess the merits of animals for New Zealand outside our own environment. Obviously the answer to the second part of this question is that the reason we want to bring the animals here is to test them under New Zealand conditions. But we can make the decision as to which exotic breeds are worth testing here, by looking at situations overseas where sheep are kept under conditions similar to our own and have production records better than any sheep in New Zealand. One of the best examples known to the writer is that of a small flock of Oldenburgh sheep on the farm of R. Ragendanz, a breeder of Kent sheep near Rye in England. Comparative figures for percentages of lambs born for the Oldenburghs and the Kents, respectively, are: Mature ewes, 182 and 145; two-tooths, 178 and 120.

As well as the reproductive advantage, the mature Oldenburgh ewes gave about 1 kg more wool of the same type per year, most of the Oldenburgh hoggets conceived to matings in their first autumn, and the Oldenburgh lambs grew much faster and much bigger than their Kent contemporaries on the same farm. It would therefore appear that, under the best possible conditions for comparison outside of New Zealand, there is a sheep that is very similar to the New Zealand Romney that is being out-produced

by another breed. It seems obvious that we must look more closely at the Oldenburgh sheep in New Zealand.

Introducing exotic breeds really has two purposes. One is to do better than we are doing now, the other is to introduce new characters so as to make the sheep industry more flexible in the face of changing conditions. One example of the first aim would be that, if higher fertility was thought to be a desirable characteristic, we could either select within our present breeds or import high fertility sheep from overseas for crossbreeding. The approach to the question depends on the sheep with which change is desired.

Quinlivan *(loc cit.)* asserts that "by careful selection of Romney sheep it should be feasible to have a 200% lamb drop without much difficulty". The only published data on selection in Romneys for fertility is that of Wallace (1964). In that experiment, the percentage of lambs born in the flock selected for fertility had increased to 22% above that of the control flock after 15 years of selection — about 1½% increase per year. It is suggested that it would be worth while testing the use of a very high fertility breed like the Finnish Landrace for more rapid increase in fertility. Similar examples could be given for growth rate, eye muscle area, and milk production.

An example of importing breeds for the second purpose — introducing desirable new characters — would perhaps be the strains of fine wool Merinos that thrive under high rainfall conditions.

CONCLUSION

It is obvious that the technique of ovum transport in rabbits has yet to be thoroughly tested, but the potential of this technique is sufficient to justify further experimentation. The case for importation of exotic breeds in no way depends on the use of the ovum-transfer technique, but can probably be assisted by it.

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