SUCCESSFUL LONG-DISTANCE AERIAL TRANSPORT OF FERTILIZED SHEEP OVA

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Summary. Two experiments were carried out involving the exportation by air from Great Britain of a total of three rabbit does carrying in their oviducts fertilized sheep ova for transfer to recipient ewes in South Africa. In October, 1960, ova were recovered in Cambridge from Border Leicester ewes which had been mated to a ram of the same breed. The ova were transported in two does to Pietermaritzburg, where they were transferred to Dorper ewes. In the second experiment early in December, 1960, fertilized ova were recovered from Welsh Mountain ewes (after they had been mated to Welsh Mountain rams) and finally transferred to German Merino ewes in South Africa. Following storage periods in the rabbits ranging (in the two experiments) from 101 to 128 hr, seventeen sheep blastocysts or late morulae were recovered in Pietermaritzburg and sixteen were transferred to a total of ten recipient ewes. Six pregnancies resulted and four live lambs were born to foster mothers in South Africa. The techniques are described in detail.

INTRODUCTION

This work was suggested by Averill, Adams & Rowson (1955), who reported that, following transfer to the genital tract of the rabbit, fertilized sheep ova can survive for at least 5 days and develop to the early blastocyst stage. The survival rate of the sheep ova during storage in the rabbit was rather better than that reported later for sheep ova stored *in vitro* for 3 days at 4.5 to 7.0°C (Averill & Rowson, 1959). Marden & Chang (1952) described the successful aerial transport of fertilized rabbit ova stored in a thermos flask. Storage time in their experiment was 27 hr and the temperature in the thermos had risen to 19°C at the time of unpacking. Clearly, the use of a rabbit as an 'incubator' at present offers the best prospect of successful long distance aerial transport of fertilized sheep ova.

Air transport costs for a single rabbit are low enough to make the long distance movement of livestock in this way an economic proposition. From London to Durban in Natal, for example, a charge of about £8 for carriage of a

crated 2.5 kg rabbit is made at present. The cost of transporting an adult sheep by sea from Great Britain to South Africa might be as much as £80, while these charges in the case of cattle (to which species our techniques might in future be extended) may be in the vicinity of £200 per head. The experiments reported here are the first in which the fertilized ova of farm animals have been transported in this way and subsequently developed to term.

MATERIAL AND METHODS

PLAN OF PROCEDURE

In each experiment, the dates of departure of suitable flights from London Airport determined the timing of the operations at each stage. During the periods in which the experiments were to be conducted, an air freighter was due to leave London at 2.30 a.m. on Sundays and the dates 23rd October and 4th December were selected for the departure from London. To allow time for post-operative recovery of the rabbit, the transfer of ova to the does in Cambridge was originally planned, in both experiments, for the Friday prior to the departure of the flight. In order that 4- to 8-cell sheep ova might be recovered on the Friday morning in each case, it was necessary for the donor ewes to ovulate some 24 to 30 hr previously, i.e. late on Wednesday or early on Thursday morning. On the assumption that ovulation would occur about the end of oestrus and that the latter would last approximately 24 hr, the start of oestrus in the donor ewes in Cambridge as well as in the recipient ewes in South Africa was planned for Wednesday morning, 19th October (Experiment 1) and Tuesday afternoon, 29th November (Experiment 2).

In both experiments, the oestrous cycles of the donor and recipient ewes were synchronized by means of daily injections of progesterone in arachis oil. Oestrus in the donor ewes in Cambridge was to occur during the normal autumn breeding season and synchronization at this time of year is straightforward (Hunter, 1954). In South Africa, many of the more popular breeds of sheep have breeding seasons that enable some of them to be mated in spring and early summer, particularly if the ewes have been kept on a high plane of nutrition during the previous winter (Hunter, 1961). Dorper ewes, a hardy local mutton breed developed in South Africa from crosses between the Dorset Horn and the Blackhead Persian, were available as prospective recipients for the first experiment in October and German Merinos for the second experiment in December. Synchronization during the early summer of the oestrous cycles of both these breeds had been found previously to be satisfactory (Lishman & Hunter, 1961). Because their cycle lengths are usually about 17 to 19 days, progesterone treatment of the ewes in South Africa continued for 21 days in each case and the timing of the whole synchronization treatment, particularly that of the last progesterone injection, was ultimately determined by the expected time of departure of the selected flight.

The donor ewes in Cambridge were primed with a total of 100 mg of a horse anterior pituitary preparation, AP118B, to induce superovulation. This was given subcutaneously in five equal doses at 12-hourly intervals, commencing

at different stages in the two experiments (see later). The techniques employed for the *in vivo* recovery of ova from the donor ewes in Cambridge and transfer to the recipient ewes in South Africa have been previously described (Hunter, Adams & Rowson, 1955).

The rabbits to which the sheep ova were to be transferred were given an ovulating injection (25 i.u. LH, 'Pregnyl', Organon) about 24 hr before the transfer operation. Preliminary experiments had shown that 2- to 8-cell sheep ova could survive for 4 to 5 days in the ligated oviduct of the rabbit (Adams & Rowson, 1960—unpublished data) and since a greater proportion of ova are generally recovered from the oviduct than from the uterus, immediately prior to transfer of the ova to the does their oviducts were ligated near the tubouterine junction. After arrival in Pietermaritzburg, the does were killed and the oviducts excised for flushing. Sterile sheep blood serum was used at all stages of the experiments in the processes of recovery and transfer of the ova. The rabbit carcasses were incinerated shortly afterwards, thus satisfying an important condition of the Veterinary Import Permit issued in South Africa.

Experiment 1

As their oestrous cycles were already fairly well synchronized, it was not necessary to administer progesterone for a long period to the six Border Leicester donor ewes in Cambridge. Three of the ewes received eight and three only seven daily injections of 10 mg progesterone, the last in each case being administered at about 11 a.m. on 15th October. Twenty-four hours later, treatment of these ewes with anterior pituitary extract was commenced. The ewes were then run with a raddled, fertile Border Leicester ram; two were served during 17th October and the remaining four between 5 p.m. on 17th October and 8 a.m. the following morning. In the meantime, the Dorper ewes in South Africa received daily injections of 10 mg progesterone from 26th September and for the last time at about 2 p.m. on 16th October. By the use of vasectomized rams, the ewes were thereafter tested every 2 hr for oestrus until the display in all cases was over. Of the nineteen treated ewes, fourteen commenced oestrus between 2 a.m. and 2 p.m. on Wednesday, 19th October. Two more came into oestrus by midnight and the remaining three on 20th October. Oestrus continued in these ewes for 14 to 34 hr (mean 24.1 ± 1.4 hr).

Ovum recovery was performed in Cambridge on Thursday afternoon, 20th October, instead of Friday 21st as planned. This was necessary as the prospective donor ewes had commenced oestrus rather earlier than expected. This fact possibly also explains the low ovulation rate: sixteen ova (three 1-cell, two 4-cell and eleven 8-cell) were recovered from the six Border Leicester ewes, which had a total of only eighteen ovulations. After recovery had been carried out on each ewe, the ova were stored in dialysis chambers at 37°C (Averill & Rowson, 1958) until sufficient had accumulated for transfer to a rabbit. All the ova were transferred to two rabbits, the thirteen cleaved ova being divided between three oviducts, whilst the three 1-cell ova (these were probably unfertilized) were transferred to the remaining oviduct. The details of recoveries and transfers are given in Table 1.

Information regarding the numbers and stages of ova transferred to each

TABLE

THE TIMING OF RECOVERIES AND TRANSFERS OF

CAMBRIDGE							
	Donor ewe	Oestrus commenced by	Time oviducts flushed	Ova recovered	Ova transferred		
					at	Doe	to Side
Experiment 1	20 OCTOBER						
	183	8.00 a.m. 18 Oct.	2.50 p.m.	3 1-cell	5.45 p.m.	58/28	R
	190	8.00 a.m. 18 Oct.	3.30 p.m.	5 8-cell	4.20 p.m.	58/71	L (4)
	191	8.00 a.m. 18 Oct.	3.50 p.m.	2 8-cell			R (3)
	192	8.00 a.m. 18 Oct.	4.40 p.m.	2 4-cell	5.45 p.m.	58/28	L
	195	8.00 a.m. 17 Oct.	5.05 p.m.	3 8-cell			
196	2.30 p.m. 17 Oct.	5.30 p.m.	1 8-cell				
Experiment 2	30 NOVEMBER						
	420	9.00 a.m. 28 Nov.	11.25 a.m.	{ 1 3-cell 1 2-cell	12.25 p.m.	LH/15	R
	410	9.00 a.m. 28 Nov.	11.45 a.m.	1 1-cell			
	405	9.00 a.m. 28 Nov.	12.10 p.m.	5 1-cell			
	1 DECEMBER						
	414	2.00 p.m. 29 Nov.	3.05 p.m.	1 2-cell	3.40 p.m.	LH/15	L
419	9.00 a.m. 29 Nov.	3.25 p.m.	{ 1 3-cell 6 8-cell				

All times shown are G.M.T.; local

OVA AND THE ONSET OF OESTRUS IN DONOR AND RECIPIENT EWES

PIETERMARITZBURG							
<i>Doe killed</i>	<i>Oviduct flushed</i>	<i>Ova recovered</i>	<i>No.</i>	<i>Time</i>	<i>Ova transferred Recip. ewe</i>	<i>Recipient's synchronized oestrus commenced</i>	<i>Outcome</i>
p.m. 24 OCTOBER; a.m. 25 OCTOBER							
12.55 a.m.	2.25 a.m.	3 1-cell	—	(unfertilized?)			
10.10 p.m.	10.20 p.m.	2 blastocysts	1	10.45 p.m.	101	10 a.m. 19 Oct.	Ewe died (occlusion of intestine) 2 Nov. Not pregnant
			1	10.55 p.m.	4	8 a.m. 19 Oct.	Ewe returned to service 6 Nov.
	11.25 p.m.	3 blastocysts	2	11.35 p.m.	501	8 a.m. 19 Oct.	Ewe died (lymphadenomatosis) 14 Dec. One foetus—see Plate 1.
			1	11.55 p.m.	3	10 a.m. 19 Oct.	Ewe returned to service 6 Nov.
12.55 a.m.	1.10 a.m.	6 blastocysts	2	1.20 a.m.	5	4 p.m. 19 Oct.	10.0 lb. ♂ lamb born 15.3.61 Pl. 2, Fig. 3
			2	1.30 a.m.	22	4 a.m. 19 Oct.	11.1 lb. ♂ lamb born 16.3.61 Pl. 2, Fig. 3
			1	2.10 a.m.	40	10 p.m. 19 Oct.	Ewe returned to service 27 Nov.
			1	Lost (pipette snapped during transfer)			
5 DECEMBER							
8.45 p.m.	10.00 p.m.	{ 5 1-cell 1 2-cell 1 blastocyst	—	(unfertilized?)	6	8 p.m. 29 Nov.	10.5 lb. ♀ lamb born 1.5.61 with 'parrot mouth' (Pl. 2, Fig. 4)
			—	(not viable?)			
			1	10.15 p.m.			
8.45 p.m.	9.00 p.m.	5 late morulae	1	10.20 p.m.			
			2	9.25 p.m.	214	4 a.m. 30 Nov.	10.5 lb. ♂ lamb still-born 20.4.61 following severe verminosis and anaemia
			2	9.10 p.m.	245	2 p.m. 29 Nov.	10.5 lb. ♀ lamb born 26.4.61 (Pl. 2, Fig. 4)

time in Pietermaritzburg was 2 hr ahead of Greenwich time.

B*

oviduct of the two rabbits were sent by airmail and received in Pietermaritzburg on Monday, 24th October. From among the sixteen ewes that had commenced oestrus on 19th October, eight in good condition, which had records of good fertility—i.e. had lambed regularly each year—were then selected and prepared for the transfers, the intention being to transfer not more than two ova to each recipient ewe. In the meantime, the rabbits were flown from London Airport as arranged, met at Durban Airport on Monday evening, 24th October, and brought 60 miles by car to the Ukulinga Experiment Station, Pietermaritzburg, where they finally arrived at 11.30 p.m. local time (9.30 p.m. G.M.T.).

Recovery of the ova from the first rabbit, 58/71, commenced shortly after midnight, local time (shown in Table 1 as 10.10 p.m. G.M.T.). After the right oviduct and uterine horn had been placed in a covered watchglass in an incubator set at 38°C, the left oviduct was dissected out and flushed. Two sheep blastocysts were located, one being transferred to the uterine horn of Dorper ewe No. 101 and one to ewe No. 4. The two remaining ova, which had been originally transferred to the rabbit's left oviduct, were not recovered. During the intervals between location and transfer, the watchglasses containing the ova were kept, as far as possible, covered in the incubator. The right oviduct, after storage in the incubator for an hour, was then similarly dissected and flushed and three blastocysts were located 80 min after slaughter of the rabbit. The details of their transfer to recipient ewes, together with those of the recoveries from the second rabbit and transfers of these ova to recipient ewes are given in Table 1. The times of commencement of oestrus in associated donor and recipient ewes are also shown in the Table. Because the ova recovered from two or three donor ewes were accumulated and transferred together to one rabbit oviduct, it is not possible to follow precisely the fate of ova from individual ewes. However, the origins or fates of groups of ova may be traced in the Table.

Experiment 2

The synchronization treatment of five Welsh Mountain ewes in Cambridge consisted of sixteen daily injections of 15 mg progesterone, the last being administered at about 11 a.m. on 25th November. The priming injections in this experiment commenced simultaneously with the end of the progesterone treatment. Subsequent services by raddled, Welsh Mountain rams were recorded daily at approximately 9 a.m., 2 p.m. and 5 p.m. (Table 1). The prospective recipient ewes in Pietermaritzburg (German Merinos) were given, from 6th November, twenty-one daily injections of 10 mg progesterone, the last being at about 5.30 p.m. on 26th November, and were then teased by vasectomized rams at 2-hourly intervals. One of the twenty ewes showed no sign of oestrus following this synchronization treatment. Oestrus commenced in two ewes between 4 a.m. and 8 a.m. on Tuesday, 29th November; between 2 p.m. and 10 p.m. eleven ewes first accepted service and the remaining six were picked out by the teaser rams by 4 p.m. on 30th November. The mean length of oestrus in the German Merino ewes was 18.9 ± 1.4 hr, some 5 hr less than in the Dorsers in the first experiment.

PLATE 1

FIG. 1. The chorion and contents recovered *post mortem* from Dorper ewe No. 501 (scale in cm). The ewe had been the recipient of two sheep blastocysts that had been transported from Cambridge to Pietermaritzburg in the ligated oviduct of a live rabbit. Death of the ewe was due to extensive lymphadenomatosis and occurred 8 weeks after the oestrus following which ovum transfer had taken place. Only one embryo was found *post mortem* and is clearly visible within the amnion. The embryo itself, apparently degenerating, is shown in Fig. 2 (magnification approximately $5\times$). The condition of the ewe, which deteriorated rapidly prior to her death, was presumed responsible for the mortality of the embryo after it had survived transfer.

PLATE 2

Lambs born in South Africa following their importation from Great Britain as fertilized ova. The ova were transported from Cambridge to Pietermaritzburg in the ligated oviducts of live rabbits and transferred on arrival to the uteri of local ewes, shown here with the lambs. FIG. 3. Two 5-month-old Border Leicester ram lambs (in the centre) with the Dorper ewes that reared them from the blastocyst stage. FIG. 4. Two 3½-month-old Welsh Mountain ewe lambs with their German Merino 'foster mothers'. The short or 'undershot' mandibles ('parrot mouth') of the smaller lamb on the left made both suckling and eating difficult for the lamb.

Because of the extent of the variation in time of onset of oestrus in the donor ewes, on 30th November ova were recovered from three ewes and transferred to the right oviduct of a doe and on the following day ova from two more ewes were transferred to the left oviduct of the same rabbit (Table 1). Whereas in the first experiment unexpectedly low ovulation rates occurred in the donor ewes, in Experiment 2 a high percentage of the ova recovered was not fertilized. As in Experiment 1, the recovered ova were stored in dialysis chambers until transfer to a ligated oviduct of the rabbit.

As before, advice concerning the ova carried by the rabbit was received in Pietermaritzburg on the Monday (5th December) and sufficient German Merino ewes were selected from among those with synchronized oestrous cycles. The rabbit arrived early that evening and the records of ova recovered and transferred are given in Table 1.

RESULTS AND DISCUSSION

The pregnancies resulting from these transfers are indicated in the Table. In the first experiment, the sheep ova remained in the oviducts of the rabbits for approximately 102 and 103 hr. Fourteen out of a total of sixteen ova were recovered from the two does and ten of the eleven that had continued cleavage, apparently normally, to the early blastocyst stage were transferred to seven Dorper ewes, three of which became pregnant, two eventually lambing. In the second experiment, five out of eight ova were recovered after storage in the doe's left oviduct for 101 hr and seven out of eight after storage in the right oviduct for about 128 hr. At least two pregnancies followed transfer of ova recovered from the doe's left oviduct, but it is not possible to state whether it was the blastocyst recovered from the right oviduct or the fifth morula from the left oviduct which survived storage and transfer to Ewe 6. However it may be possible to determine the parents of this lamb by blood grouping and in the future such tests may be essential if it is required to establish for stud-book purposes the parentage of animals born to foster mothers following their transfer as fertilized ova.

For efficient application of these techniques, methods for reducing the vulnerability of the ova during the two *in vitro* storage periods are necessary. The use, for example, of the dialysis chamber (Averill & Rowson, 1958), or simply of small stoppered tubes (Hancock & Hovell, 1961), for storage of the ova between killing of the rabbit and transfer to the recipient ewes, as well as during the period following recovery from the donor ewes, appears to be preferable to the covered watchglasses employed in these experiments. Presumably more lambs can be expected per rabbit if the ova can survive longer intervals of *in vitro* storage during this second period, or, alternatively, if the rate of laparotomies and transfers of ova to recipients can be increased. In these experiments the number of ova transferred to the rabbits was governed by the number of suitable ova recovered from donor ewes. If, however, the supply of ova is not a limiting factor, the number that can be transported per rabbit and survive transfer to recipients will be limited in practice by their survival following recovery from the rabbit and pending transfer to the recipient

ewes. The upper limit to the number of ova that will survive mere simultaneous storage in one rabbit remains to be determined.

These techniques are likely to be of value in cases where the importation of exotic breeds is desirable and either the cost of transportation is prohibitive, or, more particularly, where for reasons of disease control the importation of farm animals cannot be permitted. Livestock transported as embryos are not at present subject to a costly period of quarantine, but may instead, as a result of being reared by a foster mother adapted to local conditions, have the advantage of receiving some degree of passive immunity to some of the diseases of their new environment. Furthermore, some of the difficulties of acclimatization, with which adult stock imported into a new environment are faced, may also be reduced in this way. These two possibilities appear to merit investigation. On the other hand, an importer of pedigree livestock who employs these methods will, for the time being, have no say as to the sex of the imported animals and his choice in the first place will be limited to the selection of sire and dam.

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