THE DEVELOPMENT OF SHEEP EGGS IN THE RABBIT OVIDUCT AND THEIR VIABILITY AFTER RE-TRANSFER TO EWES

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Summary. A total of 456 sheep eggs at the two-, four-, eight- and sixteen-cell stages were transferred to the ligated oviducts of pseudopregnant or oestrous rabbits and 397 (87%) of the eggs were recovered. Of these, 368 (93%) continued development in the rabbit. The proportions of eggs recovered and of eggs developing were not affected by the cleavage stage at the time of transfer to the rabbit or by the endocrine status of the rabbit. Development in the rabbit oviduct appeared to proceed normally up to the blastocyst stage. In many instances, although rupture of the zona pellucida occurred, complete escape from it appeared to be frustrated by the 'mucin coat' acquired in the rabbit's oviduct.

Sheep eggs recovered from the rabbit's oviduct were re-transferred to ewes which had been in oestrus within 24 hr of the original donor ewes. The highest survival rates to lambing were obtained when eggs were re-transferred to recipient ewes after 3 days in the rabbit. The best rate of survival (69%) was with two- and four-cell eggs which had been in pseudopregnant rabbits for 3 days.

The proportion of the re-transferred eggs which survived declined when the period in the rabbit oviduct was extended from 3 to 5 days, and was negligible after 7 days. The results are discussed in relation to the pattern of embryonic death observed in the recipient ewes.

In order to test whether the lessened viability of such eggs resulted from retardation of their development, sheep eggs recovered from oestrous rabbits were also re-transferred to recipient ewes which had come into oestrus 48 to 60 hr later than the original egg donors. The survival rate of such eggs was not improved.

A further fifty-one one-cell eggs recovered from donor ewes on Day 2 were transferred to the ligated oviducts of oestrous rabbits. All of the eggs in which there had been some evidence of fertilization continued to develop, some as far as the blastocyst stage.

It was concluded that the oviducts of either oestrous or pseudopregnant rabbits provide a satisfactory environment for the development of

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sheep eggs from the one-cell stage blastocysts, but the viability of such eggs declines rapidly after more than 5 days in the rabbit.

INTRODUCTION

Development of methods of storing fertilized eggs is a prime requirement before wider applications of the technique of egg transfer to animal breeding are practicable. So far, attempts to store eggs in a state of arrested development have had only limited success (Averill & Rowson, 1959; Harper & Rowson, 1963; Hancock, 1963; Buttle & Hancock, 1964; Kardymowicz, Kardymowicz & Kremer, 1966). Culture *in vitro* of sheep eggs from early cleavage stages has also proved to be difficult and the viability of such eggs after re-transfer is poor (Wintenberger, Dauzier & Thibault, 1953; Hancock, 1963; Moore, 1970; Moor & Cragle, 1971). Wintenberger *et al.* (1953) and Moor & Cragle (1971) have cultured sheep morulae *in vitro* to the blastocyst stage and some such blastocysts have been shown to be viable (Moor & Cragle, 1971).

Sheep eggs have been shown to be capable of further development from the early stages of cleavage to blastocysts in the oviducts of pseudopregnant rabbits (Averill, Adams & Rowson, 1955; Averill, 1956; Hunter, Bishop, Adams & Rowson, 1962) or in oestrous rabbits (Adams, Moor & Rowson, 1968). In the absence of suitable *in-vitro* methods, this technique offers a means of maintaining sheep eggs for short periods outside the ewe.

The object of the present experiment was to evaluate the relationships between the cleavage stage of sheep eggs at the time of transfer to rabbits, the time spent in the rabbit oviduct, and the subsequent viability of such eggs after re-transfer to ewes. This was done to establish the optimum conditions for the survival of such eggs after re-transfer to ewes.

MATERIALS AND METHODS

Experimental design

Experiment 1. Sheep eggs were transferred to the oviducts of oestrous rabbits in three groups according to their stage of development at the time of recovery from the donor ewes. The groups were (i) two- and four- cell eggs, (ii) eight-cell eggs, and (iii) sixteen-cell eggs. Within each of these groups, eggs were recovered from the rabbit after 3, 5 or 7 days. The rabbits were not tested for oestrous behaviour, but were mature does whose ovaries contained ripe follicles and no corpora lutea.

Insufficient animals were available to allow this design to be duplicated in a parallel series of transfers using pseudopregnant rabbits. However, to obtain an indication of the comparative suitability of the pseudopregnant rabbit's oviduct as an environment for the preservation of sheep eggs, a series of transfers of two- and four-cell eggs and of recoveries after 3, 5 and 7 days from the oviducts of pseudopregnant rabbits were also performed.

After recovery from the rabbit, the sheep eggs were examined microscopically ($\times 100$) and classified according to the stage of cleavage which they had reached. Four classes were distinguished.

- (i) Morulae, including sixteen- to thirty-two-cell eggs,
- (ii) Late morulae which included eggs which had developed beyond thirty-two-cells and up to the stage of blastocoele formation,
- (iii) Blastocysts,
- (iv) Late blastocysts in which the zona pellucida was ruptured or in which complete escape from the zona pellucida had occurred.

After completion of the microscopic examination to test their viability, a proportion of the eggs was then re-transferred to the uteri of recipient ewes which had been in oestrus within 24 hr after oestrus was first observed in the original donor.

Experiment 2. In addition to the cleaved eggs transferred in Exp. 1, some uncleaved, one-cell eggs which were recovered from donor ewes on Day 2 were also transferred to rabbits. The results of these transfers are considered separately. None of these eggs was re-transferred to a recipient ewe after recovery from the rabbits.

Experiment 3. To test whether the failure to survive in recipient ewes of some of the re-transferred eggs was due to a lack of synchronization resulting from retardation of the eggs' development whilst they were in the rabbit, a supplementary series of re-transfers was made. Eggs at the two- and four-cell and eight-cell stages were transferred to the oviducts of oestrous rabbits for 7 and 5 days, respectively, and then re-transferred to ewes which had been in oestrus 48 to 60 hr later than the original donors.

Experimental animals

A total of 238 ewes was used in three experiments described above; 109 were used as egg donors and 129 as recipients. To detect oestrus, the ewes were run with a raddled, vasectomized ram and inspected twice daily for service marks.

The donor ewes were treated with 900 to 1200 i.u. *r*MSG on Day 12 of the oestrous cycle. At oestrus, each ewe was mated with at least two different rams. Eggs were recovered from these ewes on Days 2, 3 or 4 after the onset of oestrus, using the technique of Hunter, Adams & Rowson (1955).

As soon as possible after recovery and examination, the eggs were transferred into the oviducts of rabbits. This was accomplished by taking up the eggs together with a small quantity of medium in a fine bore Pasteur pipette, the tip of which was fire-polished and bent to an angle of 120°. The pipette was introduced through the fimbria into the oviduct to a depth of 3 to 4 cm before gently expelling the eggs. Pending transfer, the eggs were held in sterile sheep serum at 37°C. After transferring the eggs to the rabbits' oviducts, a single cotton ligature was placed on the ovarian side of the uterotubal junction to prevent the passage of eggs into the uterus. To induce pseudopregnancy, does were treated with 25 i.u. *h*CG given intravenously 24 hr before egg transfer. Does used during the follicular phase were not tested for oestrus but, at the time of egg transfer, it was ascertained that there were no corpora lutea present in their ovaries.

To recover the eggs from the rabbits' oviducts, the animals were killed and bled, and the oviducts removed aseptically. The excised oviducts were flushed with 1 to 2 ml sterile sheep serum at 37° C. After microscopic examination

and classification (see Experimental Design), pairs of eggs which had reached an identical stage of development were re-transferred to the uteri of suitably synchronized recipient ewes.

After recovery from the egg transfer operation, the recipient ewes were kept with a raddled, vasectomized ram and observed daily for signs of oestrus. Early in the experiment, it became apparent that in certain cases embryonic mortality, indicated by delayed returns to oestrus, was occurring. Thereafter, all animals which did not exhibit signs of heat underwent laparotomy during the 2nd month of gestation. The uterus was palpated to determine the size and state of the conceptus, but no attempt was made to diagnose the number of fetuses present, as it was considered that excessive handling at this time could adversely affect the pregnancy. Where pregnancy was progressing normally, the ewe was allowed to recover and go to term but, if the presence of a resorbing conceptus was suspected, the ewe was killed to permit examination of the uterine contents.

RESULTS

Egg recovery from donor ewes

The numbers of ovulations (corpora lutea) in donor ewes, and the numbers and stages of development of the eggs recovered from them on Days 2, 3 or 4 after the onset of oestrus are shown in Table 1. Recovery rates were slightly

TABLE 1

RECOVERY RATES AND STAGES OF DEVELOPMENT OF SHEEP EGGS RELATIVE TO THE TIME OF RECOVERIES (NO. OF DAYS AFTER OESTRUS) FROM DONOR EWES

Day of egg recovery	No. of ewes	No. of CL	Eggs recovered		Eggs cleaved		Stage of development			
			No.	%	No.	%	2-cell	4-cell	8-cell	16-cell
2	47	329	270	82	218	81	127	75	16	0
3	37	263	216	82	191	88	1	20	159	11
4	25	230	161	70	147	91	0	0	25	122
Total	109	822	647	79	556	86	128	95	200	133

poorer on Day 4 than on Days 2 and 3. This was possibly related to the slightly higher mean ovulation rate of 9.2 in ewes on Day 4 compared with 7.0 and 7.1 ovulations per ewe on Days 2 and 3, respectively.

The proportion of recovered eggs which had cleaved was lower on Day 2 (81%) than on Days 3 and 4 (88 and 91%). Evidence that some of the un-cleaved eggs recovered on Day 2 were, in fact, fertilized is presented later.

It can be seen from Table 1 that recoveries made at these times yielded high proportions of eggs at the two- and four-cell, eight-cell and sixteen-cell stages.

Development of sheep eggs in the rabbit oviduct

Table 2 shows the results of recoveries of sheep eggs from the rabbits' oviducts. Though there were some variations between groups in recovery rates, there was no effect of the initial stage of development of the eggs, the length of time spent

in the rabbit or the condition (oestrous or pseudopregnant) of the doe. The variations in recovery rates between groups could be accounted for by occasional poor recoveries from individual oviducts.

Of the 397 eggs which were recovered, twenty-nine (7.4%) were considered abnormal. These eggs either had not cleaved or had degenerated since transfer. The proportion of eggs developing abnormally was not affected by treatment. Abnormal eggs appeared to occur more frequently in material obtained from certain donors.

TABLE 2

THE STAGE OF DEVELOPMENT OF SHEEP EGGS IN RELATION TO THEIR INITIAL DEVELOPMENT, ENDOCRINE STATE OF THE RABBIT AND THE LENGTH OF TIME THEY WERE KEPT IN THE RABBIT OVIDUCT

Initial stage of egg development	State of rabbit	Days in rabbit	No. of eggs transferred	No. of eggs recovered (%)	Stage of egg development at recovery				
					No further development	16 to 32 cells	Late morula	Blasto-cyst	Late blasto-cyst
Two- and four-cells	Pseudo-preg-nant	3	29	24 (83)	1	14	9	0	0
		5	26	21 (81)	2	0	9	10	0
		7	31	24 (77)	0	0	0	4	20
Two- and four-cells	Oest-rous	3	29	26 (90)	4	22	0	0	0
		5	30	27 (90)	0	0	11	15	1
		7	49	48 (98)	4	0	0	32	12
Eight-cells	Oest-rous	3	37	34 (92)	2	0	16	16	0
		5	67	64 (96)	9	0	2	34	19
		7	44	35 (80)	1	0	0	15	19
Sixteen-cells	Oest-rous	3	34	27 (79)	0	0	5	20	2
		5	38	36 (95)	5	0	2	8	21
		7	42	31 (74)	1	0	0	15	15
Total			456	397 (87)	29	36	54	169	109

The stages of development reached by eggs in each group of recoveries are given in Table 2, and the proportion of the recovered eggs which were at each stage of development is shown diagrammatically in Text-fig. 1. Though the eggs appeared to develop in an essentially normal manner in the rabbit oviduct, there appeared to be some effects of treatment on their cleavage rate. After two- and four-cell eggs had been in the oviducts of oestrous rabbits for 3 days, all of the eggs were early morulae (seventeen were sixteen-cell, five were thirty-two-cell) but similar eggs recovered from the pseudopregnant rabbits had progressed further (two were sixteen-cell, twelve were thirty-two-cell, nine were late morulae). No such difference between the two groups was apparent after 5 days but, after 7 days, only twelve of forty-four blastocysts recovered from oestrous rabbits were classified as late blastocysts as against twenty of twenty-four from the pseudopregnant rabbits. These differences may have been caused in part by the greater proportion of four-cell eggs transferred, by chance, to pseudopregnant rabbits (57% compared with 33%). It cannot, therefore, be definitely concluded that the rabbit's oviduct provides a more satisfactory site for the development of sheep eggs during pseudopregnancy than in oestrus.

TEXT-FIG. 1. The relative proportions of morulae, late morulae, blastocysts and late blastocysts found when two- and four-cell, eight-cell and sixteen-cell sheep eggs were transferred to the oviducts of oestrous or pseudopregnant rabbits and recovered after 3, 5 or 7 days.

Among eggs aged 7 days (post-oestrus in the donor ewe) at the time of recovery from oestrous rabbits, a higher proportion of late blastocysts was found when sixteen-cell eggs were recovered after 3 days in the rabbit than was found when eggs transferred at the two- and four-cell stages were recovered after 5 days. With eggs aged 9 days at recovery from the rabbit, there was a similar difference in the proportion of late blastocysts found when sixteen-cell

EXPLANATION OF PLATE 1

FIG. 1. A sixteen-cell egg recovered 3 days after two- and four-cell sheep eggs were transferred into the oviduct of an oestrous rabbit. $\times 235$.

FIG. 2. A late morula recovered after two- and four-cell sheep eggs had been in the oviduct of an oestrous rabbit for 5 days. $\times 240$.

FIGS. 3 and 4. Blastocysts recovered 3 days after the transfer of eight-cell sheep eggs to oestrous rabbits. The two collapsed blastocysts in Fig. 4 were expanded and similar to that shown in Fig. 3 at recovery. $\times 210$ and $\times 155$.

FIGS. 5 and 6. Late blastocysts with ruptured zonae pellucidae recovered after eight-cell sheep eggs from the same donor ewe had been in an oestrous rabbit for 5 days. Note the disparity in size between the two blastocysts. $\times 215$ and $\times 115$.

FIG. 7. Two late blastocysts photographed after they had collapsed. At the time of recovery, they were similar in appearance to the late blastocysts shown in Fig. 6. $\times 108$.

FIG. 8. A late blastocyst which has shed its zona pellucida completely. This blastocyst was recovered after eight-cell sheep eggs had been in the rabbit oviduct for 7 days. Such blastocysts in which complete escape from the zona pellucida had occurred were rarely found. $\times 100$.

PLATE I

(Facing p. 110)

eggs were recovered after 5 days and two- and four-cell eggs were recovered after 7 days. Thus, two- and four-cell eggs appeared to be retarded relative to sixteen-cell eggs as a result of the longer period for which they had been in the rabbit.

The sheep eggs recovered from rabbits were all covered by a layer of mucin which is visible in the photographs in Plate 1. The mucin layer was thicker in eggs which had been in pseudopregnant rabbits than in oestrous rabbits. Some typical morulae recovered from the oviducts of oestrous rabbits are shown in Pl. 1, Figs. 1 and 2.

At the time of recovery, sheep blastocysts which had developed in the rabbit were expanded and appeared similar to that shown in Pl. 1, Fig. 3, but they generally collapsed soon after recovery. Two collapsed blastocysts are shown in Pl. 1, Fig. 4. The mucin layer appeared to inhibit the blastocysts' escape from the zonae pellucidae and, though rupture of the zona pellucida regularly occurred (Pl. 1, Fig. 5) and blastocyst expansion continued (Pl. 1, Fig. 6), blastocysts which had lost the zona pellucida entirely were rarely encountered (Pl. 1, Fig. 8). Soon after recovery, many late blastocysts also collapsed and appeared similar to those shown in Pl. 1, Fig. 7.

It was observed that, in some blastocysts, particularly those recovered after 7 days in the rabbit, the embryonic disc appeared absent or ill defined. Sufficient material was not available to enable a detailed study to be made of such blastocysts.

Development of uncleaved eggs in the rabbit oviduct

A total of fifty-one uncleaved eggs, thirteen of which showed possible evidence of fertilization (presence of supernumerary spermatozoa and in some cases two polar bodies), recovered from donor ewes on Day 2 were transferred to the oviducts of oestrous rabbits. Eleven of the thirteen 'fertilized' eggs were recovered; all had continued to develop, some as far as blastocysts. Of the thirty-eight uncleaved eggs in which no evidence of fertilization was detectable, thirty-seven were recovered and none had cleaved whilst in the rabbits' oviducts.

Viability of eggs re-transferred to ewes from the rabbit oviduct

The results of re-transferring eggs from the rabbit oviduct to ewes whose oestrous cycles were closely synchronized with those of the original donors of the eggs are shown in Table 3. Only one of the ewes which was diagnosed as pregnant at laparotomy during the 2nd month of gestation failed to lamb. This ewe was in the group which received two- and four- cell eggs after they had been 5 days in an oestrous rabbit.

The highest survival rate (eleven of sixteen eggs) resulted when eggs, which had been transferred to rabbits at the two- and four-cell stages, were re-transferred to ewes after 3 days in the oviducts of pseudopregnant does. This survival rate was, however, not significantly better than those obtained when two- and four-cell, eight-cell, and sixteen-cell eggs were re-transferred to ewes after 3 days in the oviducts of oestrous rabbits.

After sheep eggs had been in oestrous rabbits for 5 days, the survival rates of

those which had been transferred at the two- and four-cell and sixteen-cell stages remained satisfactory (seven of twenty and nine of twenty-two, respectively) but those of eight-cell eggs (two of sixteen) and of two- and four-cell eggs which had been in pseudo-pregnant rabbits (three of twenty-one) were poor.

When sheep eggs were re-transferred after 7 days in the rabbit, only two lambs were born. Both were from eggs transferred to the rabbit at the two-cell stage; one to a pseudopregnant rabbit and the other to an oestrous rabbit.

TABLE 3

THE SURVIVAL RATES OF SHEEP EGGS RE-TRANSFERRED TO RECIPIENT EWES AFTER RECOVERY FROM THE RABBIT OVIDUCT

<i>Initial stage of egg development</i>	<i>Condition of rabbit*</i>	<i>Days in rabbit</i>	<i>No. of ewes</i>	<i>No. of eggs transferred</i>	<i>No. of lambs born</i>	<i>Proportion of eggs surviving (%)</i>
Two- and four-cell	O	3	9	18	8	44
Two- and four-cell	P	3	8	16	11	69
Eight-cell	O	3	9	18	9	50
Sixteen-cell	O	3	9	18	8	44
Two- and four-cell	O	5	10	20	7	35
Two- and four-cell	P	5	8	16	2	13
Eight-cell	O	5	11	21	3	14
Sixteen-cell	O	5	11	22	9	41
Two- and four-cell	O	7	10	20	1	5
Two- and four-cell	P	7	9	18	1	6
Eight-cell	O	7	11	21	0	0
Sixteen-cell	O	7	9	18	0	0
Total			114	226	59	

* O oestrous; P pseudopregnant.

Details of the occurrence of postoperative oestrus and the findings at laparotomy in the recipient ewes to which eggs were re-transferred from the rabbit oviduct are summarized in Table 4. The number of ewes showing oestrus after periods of less than 19 days tended to increase as the length of time eggs were kept in the rabbit was extended. Such returns would be expected if the eggs were moribund at the time of re-transfer. However, particularly in the groups receiving eggs after 5 and 7 days, there was a high incidence of returns to oestrus after periods of more than 19 days. These intervals ranged from 21 to 64 days. Such delayed returns to oestrus appear to be indicative of the occurrence of embryonic mortality, though some of those in the 30- to 36-day range could have resulted from failure to detect a previous oestrus.

Definite evidence of the occurrence of embryonic death was obtained at laparotomy during the 2nd month of gestation in those ewes which had not shown oestrus since receiving eggs. Resorbing conceptuses were identified in fourteen of the twenty-nine ewes in which pregnancy was not considered to be progressing normally. These varied from small amounts of amorphous material to well-developed fluid-filled membranes in most of which no trace of an embryo could be identified. No evidence of embryonic resorption could be found in the remaining fifteen ewes. Five of these ewes had ovulated following

egg transfer since corpora lutea were present on the ovary opposite that on which ovulation had occurred before transfer, seven had mature corpora lutea in the same ovary as at the time of egg transfer, and the remaining three ewes were anoestrous.

Lambs were born from eggs which were re-transferred from the rabbit to ewes at the morula, late morula, blastocyst and late blastocyst stages. Using these morphological criteria to classify the stage of development reached by sheep eggs when they were kept in the rabbit oviduct, it was not possible to predict the viability of eggs after re-transfer.

TABLE 4

THE NUMBERS OF EWES RETURNING TO OESTRUS AND THE FINDINGS AT LAPAROTOMY DURING THE 2ND MONTH OF GESTATION IN EWES WHICH RECEIVED EGGS KEPT FOR 3, 5 AND 7 DAYS IN THE RABBIT OVIDUCT

Days in rabbit	No. of recipient ewes	No. ewes returning to oestrus		Result of laparotomy		
		Dioestrous interval		Pregnant	No. of ewes	
		< 19 days	> 19 days		Resorbing	Barren
3	35	3	3	26	1	2
5	40	4	5	16	8	7
7	39	9	17	2	5	6
Total	114	16	25	44	14	15

Asynchronous re-transfer of eggs to recipient ewes

Fourteen sheep eggs, which had been transferred to oestrous rabbits at the eight-cell stage, were re-transferred after 5 days to seven ewes which had been in oestrus 48 to 60 hr later than the original donor ewes. One ewe gave birth to two lambs. None of eight ewes which received sixteen eggs initially at the two- and four-cell stages and which were re-transferred after 7 days in oestrous rabbits became pregnant. Resorbing conceptuses were found in two ewes in the former and three ewes in the latter series of re-transfers. There was no evidence that the survival rate of eggs kept for extended periods in the rabbit was improved by re-transferring them to ewes at an earlier stage of dioestrus than that existing when closely synchronized re-transfers were made.

DISCUSSION

In the present study, 93% (368 of 397) of sheep eggs continued to develop normally when transferred to the rabbit oviduct. The morphology of the sheep eggs which continued to develop was essentially the same as that of normal sheep eggs (Green & Winters, 1945; Rowson & Moor, 1966a). Neither the initial cleavage stage of the sheep eggs transferred to the rabbit, nor the condition of the rabbit (oestrous or pseudopregnant) appeared to affect the ability of the eggs to continue their development. Unlike the situation in culture *in vitro* (Wintenberger *et al.*, 1953; Moor & Cragle, 1971), in the rabbit's oviduct *in vivo* there appears to be no block to the development of sheep eggs during the early morula stage. It was also found that fertilized but uncleaved

sheep eggs could develop into apparently normal blastocysts in the oviducts of the oestrous rabbit. In this respect, sheep eggs also appear to differ from uncleaved mouse eggs which are unable to develop in the rabbit (Brinster & TenBroek, 1969).

It cannot be said whether the 7% of sheep eggs which failed to develop in the rabbit's oviduct did so as a result of inherent defects or because of the treatment they received before transfer to the rabbit. In this connection, it is interesting to note that Quinlivan, Martin, Taylor & Cairney (1966) found that 6 to 7% more ewes returned to oestrus before Day 17 than were considered non-pregnant on the basis of fertilization rate determined on Day 2. Possibly, the proportion of eggs which failed to develop normally in the rabbit oviduct represents those eggs which also fail under normal conditions.

The rate of cleavage of sheep eggs in the oviducts of oestrous rabbits appeared to be slightly retarded relative to that described in the normal ewe by Rowson & Moor (1966a). Such retardation could have been caused by the effects of egg transfer as demonstrated by Tarkowski (1959) with mouse eggs. However, two- and four-cell eggs appeared to develop more rapidly in the oviducts of pseudopregnant rabbits, and, though this observation is somewhat imprecise, since a greater proportion of four-cell eggs were transferred to pseudopregnant rabbits, it would seem probable that the oviduct in pseudopregnancy may provide a better environment for the developing sheep egg than that afforded by the oestrous doe. This is borne out by the better viability of eggs recovered from pseudopregnant rabbits.

Averill (1956) reported that sheep eggs kept in the oviducts of pseudopregnant rabbits until they reached the age of 10 to 13 days failed to shed the zona pellucida and began to degenerate. In both pseudopregnant and oestrous rabbits in the present experiments, however, as well as in those of Adams *et al.* (1968), many blastocysts were recovered with ruptured zonae pellucidae. Complete escape from the zona pellucida was, however, rarely observed. This process appeared to be inhibited by the layer of mucin acquired by the eggs in the rabbit oviduct. However, the mucin did not appear to restrict blastocyst growth, and though mucin is deposited more heavily in pseudopregnancy (Greenwald, 1958), the greatest proportion of late blastocysts occurred among eggs which had been in pseudopregnant rabbits.

Failure of a proportion of sheep blastocysts to rupture the zona pellucida occurred in the present experiments, and has also been observed in *in-vitro* culture (Moor & Cragle, 1971). This may indicate lack of vigour in these blastocysts. It is also possible that escape from the zona pellucida in the ewe's uterus does not depend solely on mechanical pressure from the expanding blastocyst: under normal conditions, it may be assisted by lytic factors such as have been proposed (McLaren, 1970) to exist in the mouse.

In direct, synchronous, intrauterine transfers of two eggs between ewes, egg survival rates ranging from 58 to 75% have been obtained (Averill & Rowson 1958; Moore & Rowson, 1960; Moore & Shelton, 1962, 1964; Rowson & Moor, 1966b). The survival rate of 69% (eleven of sixteen) of sheep eggs re-transferred after 3 days in the oviducts of pseudopregnant rabbits in the present experiments compares favourably with these, and shows that such sheep eggs

are little affected by these conditions. The poorer, though not significantly so, results obtained after sheep eggs had been in the oestrous rabbit for 3 days suggest that this is a less suitable environment for the development of sheep eggs.

The low viability of eight-cell eggs re-transferred after 5 days in the oestrous rabbit seems anomalous in view of their viability after 3 days, and that of two- and four-cell and sixteen-cell eggs after 5 days. Similarly, it is difficult to reconcile the poor survival rate of two- and four-cell eggs kept for 5 days in the pseudopregnant rabbit with their excellent viability after 3 days. That such eggs were not moribund is indicated by the fact that development continued when the period in the rabbit oviduct was extended from 5 to 7 days. Since two- and four-cell eggs and sixteen-cell eggs remained viable after 5 days in the oestrous rabbit, the above results must for the present be assumed to be due to chance.

The number of eggs which proved to be viable after 7 days in the rabbit's oviduct was negligible. It can, therefore, be concluded that sheep eggs are able to grow and develop in the rabbit oviduct to the stage of shedding the zona pellucida. They retain the capacity to develop normally if re-transferred into the ewe's uterus after 3 days in the rabbit oviduct but, after 5 days, their viability declines rapidly. These results are in keeping with those of Wintenberger-Torres (1956) who found that sheep eggs could develop outside the ewe's uterus, in this case in the ewe's oviduct, until Day 7 but, thereafter, development was retarded, though the eggs remained viable provided they were allowed to enter the uterus by Day 9.

The pattern of delayed returns to oestrus in ewes to which eggs were re-transferred, and the finding of resorbing conceptuses in some ewes, particularly those which received eggs which had spent 5 or 7 days in the rabbit, indicates that the sheep eggs suffered progressive damage as the period outside the ewe was increased. This damage resulted in death at a later stage of development. More specific experiments are necessary to study the aetiology of such embryonic death. Subjective observations suggested that the earliest signs of affected development may be discernible in the embryonic disc. In many resorbing conceptuses, well-developed extraembryonic membranes were found but embryonic remains could not be identified. Possibly, the embryonic tissues suffer preferential damage under these conditions. The development of trophoblastic vesicles lacking embryonic discs has been reported by Adams (1970) in rabbit eggs after culture *in vitro*, and by Tarkowski (1962) in rat eggs re-transferred after spending 3 to 4 days in the mouse.

The results of the present experiments also show that the rabbit oviduct can be used as a test system to diagnose whether one-cell sheep eggs have undergone fertilization. Such a system could be of value in assessing the results of attempts to fertilize eggs *in vitro* since the ability of such eggs to undergo normal development can be observed. For the preservation of eggs for later re-transfer, the rabbit oviduct appears to provide a satisfactory environment for up to 5 days. It seems that for longer periods of egg survival outside the ewe, techniques of storing eggs in a state of arrested development must be developed. There appear to be no marked advantages from the use of pseudopregnant rabbits in pre-

ference to oestrous does for maintaining sheep eggs, nor from the selection of particular stages of cleavage for transfer to the rabbit. In view of their viability after 7 days in the rabbit oviduct, it may, however, be advantageous to select the earliest cleavage stages when longer periods of storage in the rabbit are desired.

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