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Development of the sheep conceptus during the first fourteen days

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INTRODUCTION

Some of the most interesting changes in the development of the sheep conceptus occur within the first 14 d after mating. During this period the egg becomes fertilized, undergoes cleavage, loses its zona pellucida and forms a blastocyst which then elongates rapidly. Moreover, it has been shown that on day 12 or 13 the conceptus is capable of affecting the corpus luteum in such a way as to convert it from a corpus luteum of the cycle to the more permanent corpus luteum of pregnancy (Moor & Rowson, 1964; Moor, 1965; Moor & Rowson, 1966*a*, *b*).

The early stages in the development of the sheep conceptus have been described by several investigators, but in general with the use of relatively small numbers of animals. Assheton (1898) examined forty conceptuses obtained from sheep slaughtered 2–11 d after mating. His work was later extended, first by Clark (1934) to cover stages up to the formation of the primitive streak and then by Green & Winters (1945) who described all stages in the prenatal development of the sheep. More recent work has been confined to studies on the maturation process (Berry & Savery, 1958) and the cleavage stages during the first 5 d after oestrus (Moore, 1959; Hancock & Hovell, 1961).

During the course of experiments involving conceptus transfer (Moor & Rowson, 1966*a*) it has been possible to examine large numbers of conceptuses obtained from donor ewes between the 5th and 14th d after oestrus. This paper describes the development of such conceptuses obtained from animals in which the onset of oestrus was known to within ± 8 h. It also includes some observations on the viability of conceptuses of variable size but similar age following transfer to recipient ewes.

MATERIAL AND METHODS

The donor sheep were 177 ewes of the Dorset Horn (14), Suffolk (30) and Welsh Mountain (133) breed. Each animal was mildly superovulated with an injection of whole pregnant mares serum (800 i.u. to Welsh and 1000 i.u. to Suffolk ewes) given on the 12th d of the cycle.

Eighty-six animals were operated on for removal of the conceptus between the 5th and 9th d of the cycle and ninety-one between the 12th and 14th d. The age of all conceptuses was related to the day of onset of oestrus defined as day 0 even though ovulation normally takes place 24 h later.

Conceptuses were recovered using the techniques described by Moor & Rowson (1966*a*) up to the 9th d of development, but because of the rapid increase in size

during the later stages a different technique had to be employed for the recovery of conceptuses at ages greater than the 9th d. This technique involved the clamping of the cervix with bowel forceps to prevent escape of the flushing medium. The needle of the flushing syringe was then inserted into the tip of one horn and 10 ml of serum injected to clear any conceptuses from the ovarian section of that uterine horn. A small incision was then made into the tip of this horn through which a 3 mm bore cannula was passed. With the cannula in position a further 30 ml of serum was gently flushed through the whole uterus in the reverse direction by inserting the needle of the flushing syringe into the tip of the opposite horn. Conceptuses 11 d of age or older could readily be seen in the flushings. With the 12–14 d conceptus considerable entanglement of the elongated chorionic sac occurred during recovery and necessitated careful manipulation under the dissecting microscope to disentangle them without causing damage.

Conceptuses recovered on days 12, 13 and 14, during which period the chorionic sac rapidly elongates, were drawn into small bore pipettes (3 mm internal diameter) and their overall length measured. They were then placed in a serum-filled watch-glass for photography. Earlier stage conceptuses were placed in serum in cavity slides for detailed examination and photography.

RESULTS

A total of 602 eggs or conceptuses was flushed from the uteri of the 177 ewes between the 5th and 14th day of the cycle and of these 521 were recovered as developing conceptuses and 81 as unfertilized eggs.

Table 1 shows the ovulation rate based on the count of corpora lutea; the number of conceptuses recovered; and the number of normally developing conceptuses at six selected stages between the 5th and 14th d of the cycle.

Table 1. *The number of eggs and conceptuses recovered from donor ewes in relation to the number of eggs shed (number of corpora lutea)*

Oestrus to egg or conceptus	No. of donor ewes	No. of corpora lutea	Total no. of eggs and conceptuses recovered	No. of developing conceptuses		
				Total	Intact	Damaged
5	20	165	115	98	98	0
7	29	185	96	86	86	0
9	37	211	142	103	102	1
12	10	41	21	21	19	2
13	38	282	123	114	62	52
14	43	262	105	99	42	57
Total	177	1146	602	521	409	112

Of the total of eighty-one unfertilized eggs forty-seven were recovered from ewes showing an exaggerated response to gonadotrophin to the extent that more than twenty ovulations occurred per animal, a factor known to be associated with a low percentage of fertilized eggs. Excluding these overstimulated animals the fertilization rate in the remaining ewes was 93·4%.

Development of the fertilized eggs. There are three main phases in the development of the fertilized egg over the period studied: (1) the morula (112–128 h), (2) the blastocyst (128–224 h), and (3) the phase of elongation (day 12, 13, 14).

Phase 1. *The morula.* A total of ninety-seven morulae were recovered and of these ninety-five were obtained between the ages of 112–128 h post oestrum. The remaining two were recovered from an animal 160 h after oestrus.

An attempt was made to classify the morulae according to the number of blastomeres. Of the ninety-seven recovered morulae, thirty were in the 16–32 cell stage and sixty-seven in the 32–64 cell stage. In the case of the larger morulae the blastomeres appeared to vary in size and this became more pronouced in the early blastocyst stage.

Table 2. *The cleavage stage of conceptuses recovered from ewes 112–224 h after the onset of oestrus*

Oestrus to egg or conceptus recovery (h)	No. of donor ewes	No. of corpora lutea	Cleavage stage of recovered conceptuses					
			No. of unfertilized eggs	Morulae		Blastocysts		
				16–32 cell	32–64 cell	Zona present	Zona lost	Spherical blastocoele
112–128	20	165	17	30	65	3	—	—
160–176	29	185	10	—	2	46	26	12
208–224	37	211	39	—	—	10	8	85
Total	86	561	66	30	67	59	34	97

Phase 2. *The blastocyst.* The development of a central cavity which was the criterion of this classification was seen in 190 conceptuses recovered from 128 to 224 h after oestrus. Three substages of development could be seen within this classification:

(1) Blastocysts still within the intact zona pellucida.

(2) Blastocysts of somewhat similar size but having lost their zona pellucida.

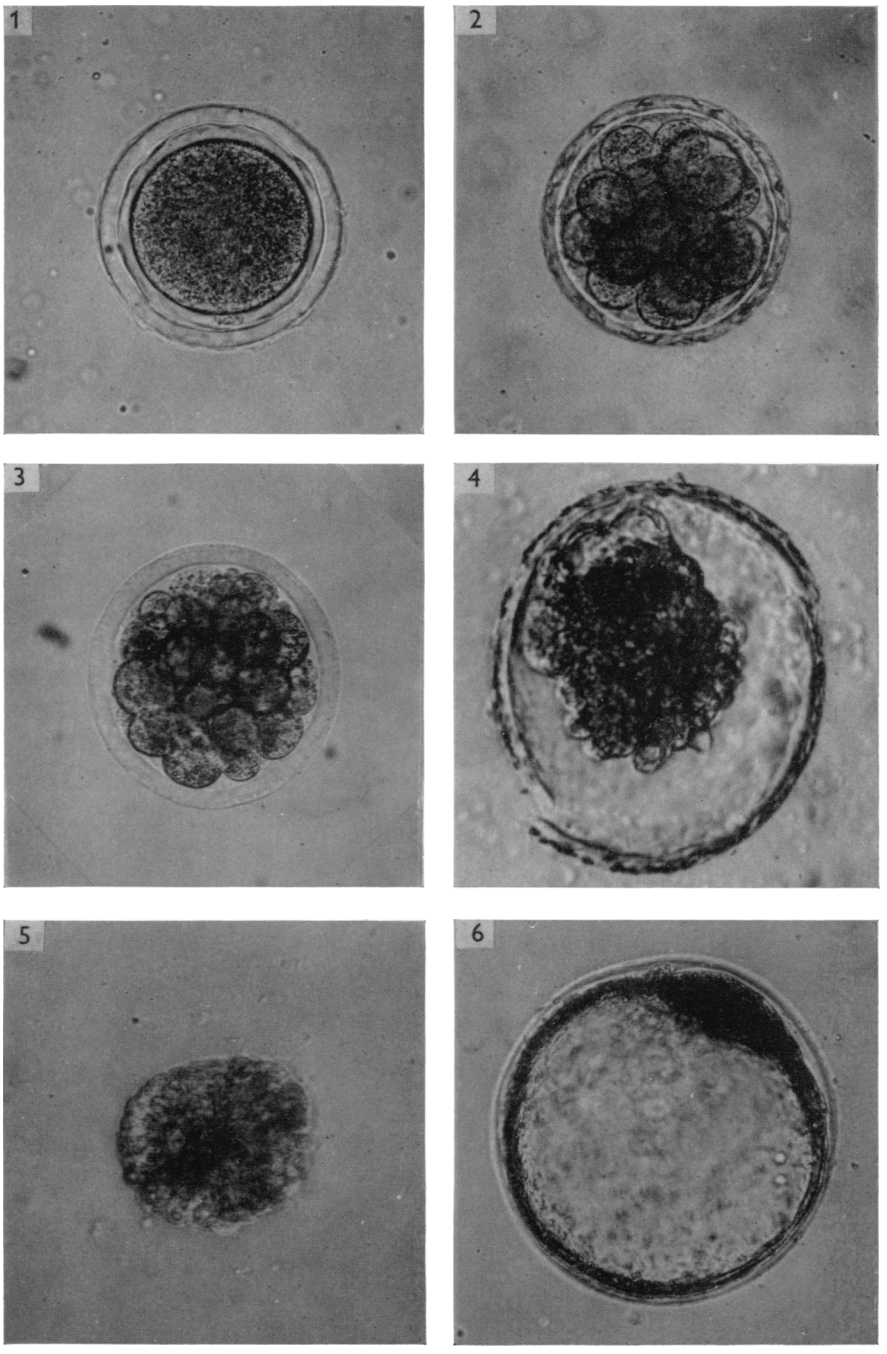
(3) Blastocysts without zona and of a larger size indicating further development from types 1 and 2.

Three blastocysts were recovered at the earliest time of this phase, 128 h after oestrus and the zonae of all three were intact although considerably thinned. By 176 h post oestrum 79% of conceptuses (38 out of 48) had lost their zonae and by 224 h post oestrum 90% (93 out of 103) were without zonae.

By 208 h post oestrum the blastocysts had enlarged and distended and showed a prominent embryonic disc. In the latest stages of this phase, however, the blastocysts appeared to adopt a collapsed and sometimes slightly wrinkled form.

Phase 3. *Elongation of the conceptus.* Recovery of conceptuses during this phase of development, i.e. days 12, 13 and 14, inevitably resulted in damage to some, but of the 234 conceptuses altogether recovered from 91 ewes, 123 were in an undamaged condition and these will be described.

At about the 12th day post oestrum the sheep conceptus commences to elongate extremely rapidly (Fig. 13) and within a period of 24 h changes from a blastocyst of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about 1 mm in diameter to an elongated chorionic sac with a mean length of 11·7 mm, in which the embryo shows up clearly as an elevated translucent oval emminence. This rapid elongation is continued through days 13 and 14, the mean size being 35·1 mm on day 13 and 102·3 mm on day 14. During this elongation process, however, the embryonic disc itself only slightly increases in size.

Table 3. *The relationship between the severity of damage to embryonic membranes and the ability of conceptuses to develop after transfer to non-pregnant recipient ewes on the 12th d of the cycle*

Recipient ewe no.	Post-oestrum stage (d)		Description of the conceptuses		Development of conceptuses
	Donor	Recipient	Length of conceptuses (mm)	Severity of damage to embryonic membranes	
1	13	12	30	Small tear in membranes	Full term
2	13	12	35	Small tear in membranes	Full term
3	13	12	45	Small tear in membranes	Full term
4	13	12	34	4 mm membrane removed from one end	Full term
5	13	12	65	15 mm membrane removed from one end	Full term
6	13	12	26	3 mm membrane removed from each end	No development

Table 4. *The number of successful and unsuccessful transfers shown in relation to the length of the conceptuses transferred*

Post oestrum stage (d)		No. of recipient ewes	Length of the transferred conceptuses (mm)							
			Successful transfers				Unsuccessful transfers			
Donor	Recipient		< 10	10–20	20–40	40+	< 10	10–20	20–40	40+
13	12	12	5	3	0		0	3	1	
13	12	12		1	5	2		1	2	1
13	13	18		0	3	1		2	8	4
Total		42	5	4	8	3	0	6	11	5

Fig. 1. Unfertilized egg, recovered 120 h after oestrus. Note the dispersion of granular material within the vitellus, and the absence of spermatozoa in the zona pellucida. × 200.

Fig. 2. A typical 32-cell morula recovered from a sheep 120 h after oestrus. × 200.

Fig. 3. A 64-cell morula, recovered 128 h after the onset of oestrus (late morula stage). The blastomeres, being of varying sizes, are thought to indicate the earliest signs of cell differentiation. × 200.

Fig. 4. An early blastocyst (168 h post oestrum) in the process of losing its zona pellucida. × 200.

Fig. 5. A blastocyst (zona lost) 170 h after oestrus. × 200.

Fig. 6. Blastocyst recovered 176 h after oestrus; showing clear disc and blastocoele formation. × 200.

Pregnancies obtained following the transfer of damaged conceptuses. Where the extent of damage to conceptuses of 13 d of age was relatively small transfers were carried out in order to determine whether such damage would effect the subsequent development. Table 3 gives the results obtained from these transfers and it can be

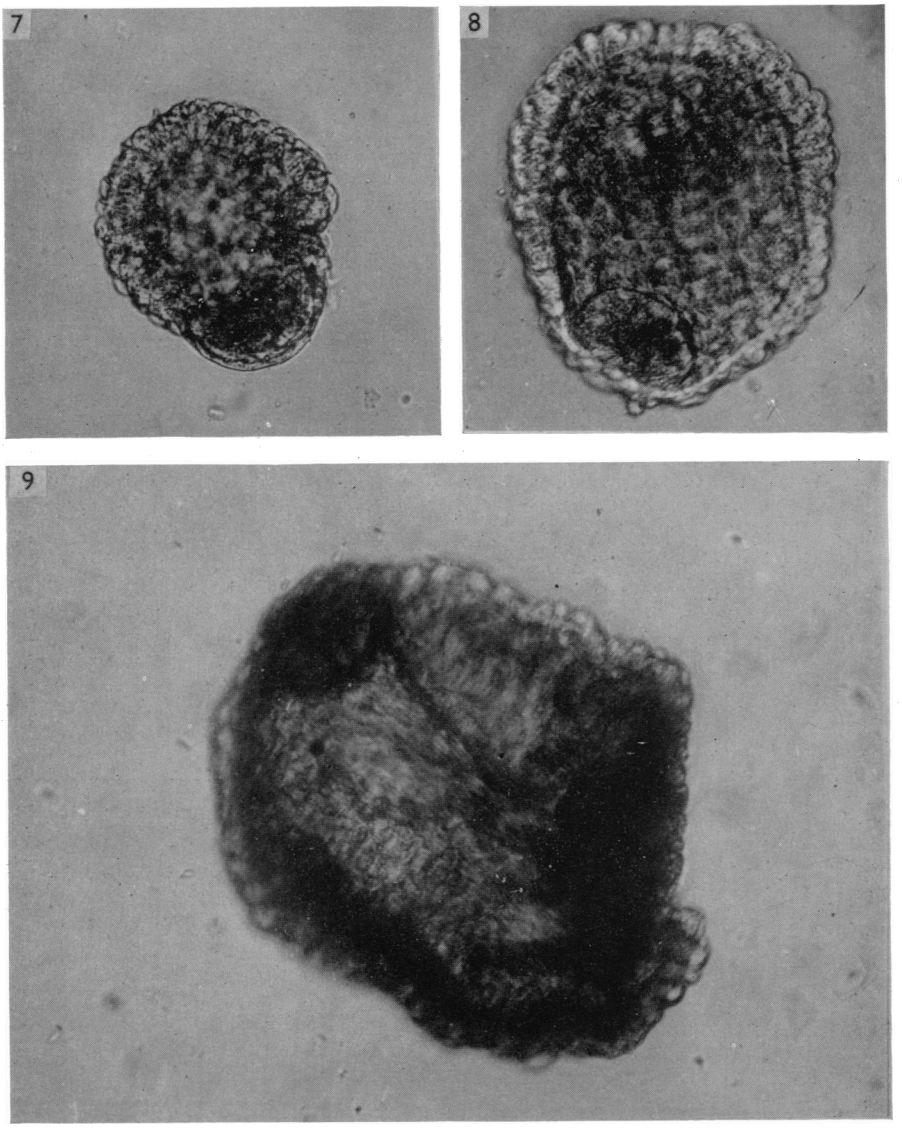


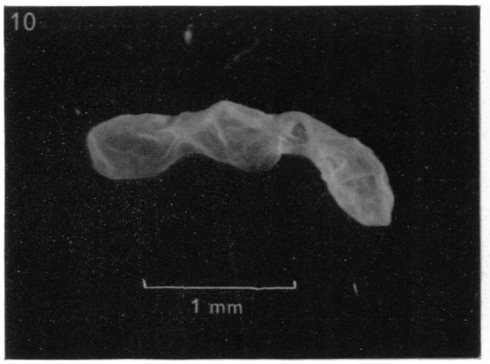
Fig. 7. Living blastocyst without zona pellucida, recovered 176 h post oestrum. × 200.

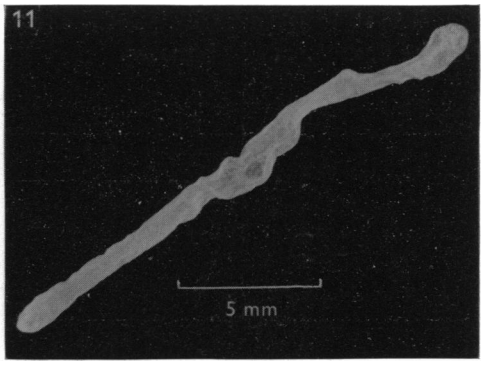
Fig. 8. Considerably enlarged blastocyst recovered from uterus of a sheep 208 h after oestrus. The disc and cavity are clearly formed.

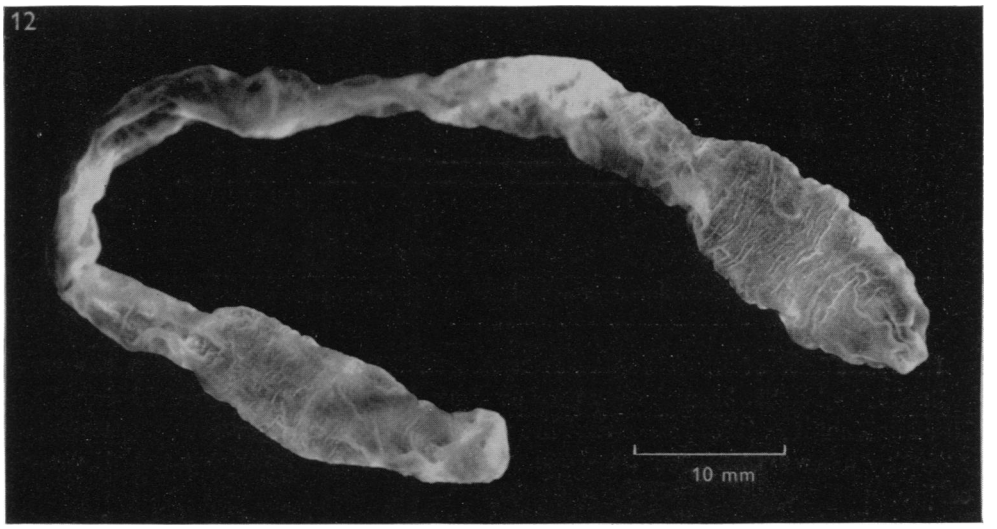
Fig. 9. An advanced blastocyst recovered 224 h after oestrus. The rapid proliferation of cells has outstripped the accumulation of fluid in the blastocoele, with resultant 'folding' of the walls of the blastocyst. × 200.

seen that some degree of damage could be well tolerated when such conceptuses were transferred to suitable recipients.

Pregnancies in relation to conceptus size. Where conceptuses the same age but of greatly differing size were obtained from the same donor and were transferred to suitable recipients they all developed equally well, irrespective of size.







Figs. 10, 11. Blastodermic vesicles of the sheep at the beginning of elongation (12d post oestrum). The embryonic disc (particularly clear, Fig. 11) is seen as a clear oval area on the membranes.

Fig. 12. Blastodermic vesicle recovered 14d after oestrus measuring 112 mm in length.

DISCUSSION

Any possible doubt as to whether the conceptuses illustrated in the figures are degenerating has been overcome by the fact that each of the developing ones shown subsequently continued to term following transfer to a suitable recipient.

The study of these conceptuses has been divided into the three phases: (1) morula, (2) blastocyst, and (3) phase of elongation as defined by Green & Winters

(1945) and Boyd & Hamilton (1952), although it is obvious that a merging of one phase into another must occur at certain stages of development.

The counting of blastomeres within the morula cannot be carried out with any great degree of accuracy and only gross differences in blastomere numbers can be indicated. In this study, therefore, the morulae were called either 'early' or 'late' depending on the state of their development. During the change from the early to the late state the overall size of the morula alters very little in spite of the increase in the number of blastomeres.

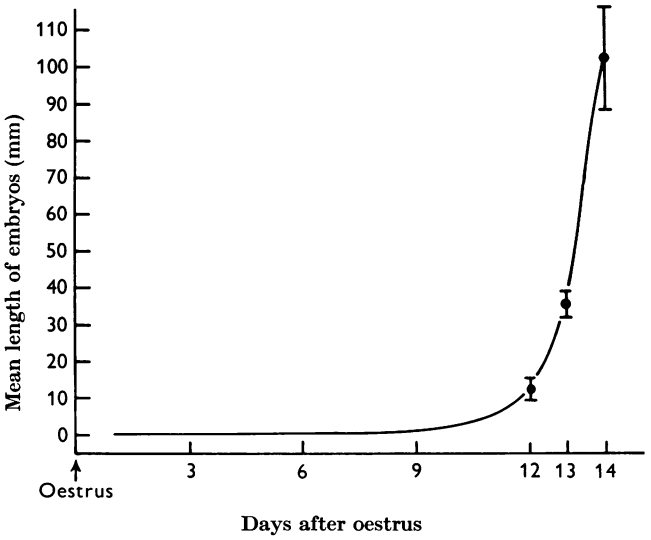


Fig. 13. Mean linear growth of the conceptus during the first 14d.

In about half of the conceptuses recovered 160–176 h after oestrus the zona pellucida had either been already shed or was split and in the process of being shed. Boyd & Hamilton (1952) state that the process of shedding does not occur until the 10th day after mating, but from our observations it would appear that it occurs earlier.

Most striking is the phenomenal growth of the conceptus which occurs from the 12th to 14th day post oestrus and this may well be related to the fact that rapid development commences at exactly the stage when the corpus luteum becomes dependent on the presence of a conceptus within the uterus for its continued maintenance.

The recovery of several conceptuses of differing size from the uteri of individual sheep provided an opportunity to test their viability when removed from an environment of competition and transferred to the uteri of suitable recipients. These experiments indicated that conceptus size played little part in their survival following transfer although it is known that had all remained within the uterus of the donor animal many would have degenerated and died. It would appear from these and other experiments that the sheep conceptus has the intrinsic ability to develop

normally until about day 11, even in such an unphysiological environment as the rabbit oviduct (Averill, Adams & Rowson, 1955) but that from this point onwards the uterus plays a more vital part in its subsequent survival.

SUMMARY

A total of 602 eggs or conceptuses was removed from the uteri of sheep during the period of from 5 to 14 days post oestrus and these are described in detail. During this period three main phases are encountered: (1) morula, (2) blastocyst, (3) phase of elongation. All conceptuses illustrated were transferred to the uteri of recipient ewes to test viability.

The transplantation of conceptuses of greatly varying size, recovered from the same ewe, into the uteri of suitable recipients, indicated that in the absence of competition the smaller conceptuses developed equally as well as the large, although had they remained within the uterus of the donor animal many of them would have died. The possible relationship of the phase of rapid elongation (day 12–13) to corpus luteum maintenance is outlined.

REFERENCES

Assheton, R. (1898). The segmentation of the ovum of the sheep with observations on the hypothesis of a hypoblastic origin of the trophoblast. *Q. Jl microsc. Sci.* **41**, 205–262.

Averill, R. L. W., Adams, C. E. & Rowson, L. E. A. (1955). Transfer of mammalian ova between species. *Nature, Lond.* **176**, 167.

Berry, R. O. & Savery, H. P. (1958). A cytological study of the maturation process of the ovum of the ewe during normal and induced ovulation. *Proc. Symp. Reprod. Infertil.* **3**, 75–82. New York: Pergamon Press.

Boyd, J. D. & Hamilton, W. J. (1952). Cleavage, early development and implantation of the egg. *Marshall's Physiology of Reproduction*, vol. II, 1–110. Ed. A. S. Parkes. London: Longman, Green.

Clark, R. T. (1934). Studies on the physiology of reproduction in the sheep. 11. The cleavage stages of the ovum. *Anat. Rec.* **60**, 135–159.

Green, W. W. & Winters, L. M. (1945). Prenatal development of the sheep. *Tech. Bull. Minn. agric. Exp. Stn*, **169**, 1–36.

Hancock, J. L. & Hovell, G. J. R. (1961). Transfer of sheep ova. *J. Reprod. Fert.* **2**, 295–306.

Moor, R. & Rowson, L. E. A. (1964). Influence of the embryo and uterus on luteal function in the sheep. *Nature, Lond.* **201**, 522–523.

Moor, R. M. (1965). The corpus luteum of the sheep. Thesis, Cambridge University.

Moor, R. M. & Rowson, L. E. A. (1966*a*). The corpus luteum of the sheep: functional relationship between the embryo and corpus luteum *J. Endocr.* **34**, 233–239.

Moor, R. M. & Rowson, L. E. A. (1966*b*). The corpus luteum of the sheep: effect of removal of the embryo on luteal function. *J. Endocr.* **34**, 497–502.

Moore, N. (1959). Studies on the reproduction of sheep. Thesis, Cambridge University.