THE TIME COURSE OF EMBRYONIC RESORPTION IN THE EWE

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Abstract

Groups of ewes were autopsied at intervals after colchicine-induced embryonic death, or at the time of return to service. Pregnant ewes treated with colchicine on days 13, 15, 17, and 19 returned to service after intervals of 7·0, 10·3, 18·2, and 21·0 days respectively.

Colchicine treatment was followed by rapid embryonic degeneration such that within 2 days fragmentation of the embryo and its membranes had advanced to a stage where cellular integrity had been lost. Resorption of the degenerate embryonic remains was a slower process which was associated with a marked leucocytic invasion of the uterine lumen.

Eight of 16 colchicine-treated ewes which were allowed to return to service did so while some embryonic debris remained *in utero*, and all 16 had uteri which were characterized by abnormally large caruncles (for oestrous ewes), the surface epithelium of which was commonly eroded.

Corpora lutea were maintained normal in weight and cellular content as long as appreciable embryonic debris remained in the uterus. That is, delayed return to service after embryonic death resulted from delayed resorption of embryonic debris rather than from any abnormality of ovarian function.

I. INTRODUCTION

Although up to 25% of ova shed by the ewe may be lost through early embryonic mortality (Edey 1969), little is known of the time course of the resorption (as opposed to abortion, which is apparently rare in early gestation) of the resultant embryonic debris. Edey (1967) showed that embryonic death before about day 12 did not interfere with normal cyclic oestrous activity, but that as age at death increased thereafter, the interval to return to service became extended and variable. The aim of the current experiment was to relate time of embryonic death to the rate of resorption of embryonic debris and to conditions in the uterus at the time of return to service.

II. MATERIAL AND METHODS

Sixty-five mature Merino ewes were placed with raddled vasectomized rams on February 13, 1970. After exhibiting one normal oestrous cycle the ewes were mated to fertile rams. Checks for oestrus and returns to oestrus were made at 0900 and 1700 hr daily. At the times indicated in Table 1 the ewes were subjected to mid-ventral laparotomy. Those ewes which possessed two or more corpora lutea were excluded from the experiment; ewes with a single corpus luteum were randomly allocated in order of mating date to one of four experimental groups (each of 10 ewes) or to a control group (of 14 ewes). Within each experimental group, four ewes were randomly selected to be retained for autopsy at the time of return to service, and three pairs for autopsy at intervals between colchicine treatment and return to service (estimated from Edey 1967), as indicated in Table 1. Randomly selected pairs of control ewes (untreated) were autopsied at times corresponding to the autopsy dates of the experimental groups.

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The embryos of experimental ewes were killed at the time of laparotomy by the injection of 0·15 mg colchicine in 0·2 ml distilled water directly into the lumen of each uterine horn. All possible precautions were taken to avoid bacterial contamination of the uterine lumen.

Ewes were autopsied either as indicated in Table 1 or on the day of return to service. Uteri were recovered and stored in warm saline before being examined for embryos and embryonic debris within 10 min of death. Heartbeat, degree of vascularity, membrane length, embryo crown–rump length, and the dry matter content of the uterine lumen (uterus flushed with distilled water and flushings dried for 24 hr at 60°C) were used to characterize all embryos recovered. In the case of embryonic debris, subjective descriptions and photographic records were made *in utero* and after recovery from the uterus.

Samples from the proximal and distal ends and from the centre of each uterine horn were taken and prepared for histological study. Whenever possible samples of embryos and embryonic membranes were also collected for histological evaluation, the membranes being prepared by the methods of Moog and Lutwak-Mann (1958). Smears were prepared from the fresh lumenal contents of each ewe to enable a check for bacterial contamination to be made, and corpora lutea were recovered, weighed, and processed by the methods of Thwaites and Edey (1970).

III. Results

(*a*) *Returns to Service*

Oestrous cycle length in non-pregnant control ewes averaged 17·4±0·3 days. As colchicine administration to pregnant ewes was progressively delayed from day 13 to day 19, the interval to returns to service was progressively extended from 7·0 to 21·0 days (Table 1).

Table 1

DAY OF AUTOPSY AND CYCLE LENGTH OF EWES RETURNING TO SERVICE IN RELATION TO COLCHICINE TREATMENT

Day of colchicine* treatment	Day of autopsy†	Mean cycle length ± S.E. (days)‡
13	14, 15, and 17	20·0±1·3
15	17, 19, and 21	25·3±1·1
17	19, 21, and 25	35·2±0·9
19	21, 25, and 30	40·0±3·8
Controls	14, 15, 17, 19, 21, 25, and 30	

* Day 0 = day of oestrus. † Two ewes per group. ‡ Four ewes per group.

(*b*) *Normal Embryos*

For comparative purposes, Table 2 summarizes the observations which were made on the embryos and corpora lutea of control ewes autopsied at intervals between days 14 and 30. With only two ewes autopsied at each stage, the weights and cellular contents of the corpora lutea did not differ significantly, and membrane lengths increased significantly only between days 25 and 30. Both embryo crown–rump

length and embryonic dry matter increased markedly with time ($P < 0{\cdot}01$), and a regular heartbeat was apparent in all embryos which were 19 days or older.

TABLE 2

CHARACTERISTICS OF THE EMBRYOS AND CORPORA LUTEA OF NORMAL MERINO EWES

All values represent the mean of two observations

Parameter	Day of autopsy						
	14	15	17	19	21	25	30
Membrane length (cm)	40·5	41·2	44·0	47·5	47·5	47·7	57·7
Crown–rump length (mm)	—	—	—	3·2	5·5	11·2	19·5
Heartbeat	No	No	No	Yes	Yes	Yes	Yes
Embryonic dry matter (g)	0·002	0·004	0·024	0·035	0·056	0·116	0·285
Corpus luteum weight (g)	0·8112	0·7387	0·8027	0·5910	0·7572	0·6935	0·7552
Type 4 lutein cells (%)	3·3	0·4	2·3	1·4	2·9	4·5	1·6

(*c*) *Day 13 Colchicine Treatment*

The embryos of ewes treated with colchicine on day 13 degenerated rapidly, though visibly detectable fragments of debris were present in the uteri of four of the six ewes autopsied on days 14, 15, and 17 (Table 3). The dry matter content of the

TABLE 3

CHARACTERISTICS OF THE EMBRYOS AND CORPORA LUTEA OF EWES AUTOPSIED AT INTERVALS AFTER COLCHICINE TREATMENT ON DAY 13

Day of autopsy	Presence of embryonic debris*	Membrane length (cm)*	Membrane cell morphology*	Corpus luteum weight (g)†	Embryonic dry matter (g)†
14	Nil, S	—, —	D, —	0·9182	<0·001‡
15	S, S	0·5, —	P.N., D	0·6957	<0·001
17	Nil, S	—, —	D, D	0·3272	<0·001

* Individual data from two ewes autopsied on each day. S, small amount of embryonic debris present; D, degenerate; P.N., pycnotic nuclei.

† Mean of two observations. ‡ Lower limit of sensitivity of technique.

uteri of each of these four ewes was, however, less than 1 mg, the limit of sensitivity of the technique used. Microscopic examination of this debris revealed that in only one case (one ewe autopsied on day 15) were recognizable cellular structures present. The weights and type 4 (degenerate) lutein cell contents of the corpora lutea of day 13 colchicine-treated ewes autopsied on days 14 and 15 were comparable to their normally pregnant controls, but in the two ewes autopsied on day 17 there was a marked (though non-significant) reduction in corpus luteum weight and a significant increase in the proportion of type 4 lutein cells.

The four ewes treated with colchicine on day 13 and allowed to return to service did so at times corresponding to cycle lengths of 18, 19, 19, and 24 days (mean $20{\cdot}0 \pm 1{\cdot}3$), but none of these contained either macro- or microscopically recognizable embryonic debris *in utero* at autopsy and the dry matter contents of their uteri were each less than 1 mg (Table 4). The corpora lutea of these four ewes were small, pale, and contained many type 4 lutein cells; one ewe had recently ovulated and the other three each possessed a large pre-ovulatory follicle.

TABLE 4

EMBRYONIC DEBRIS AND OVARIAN STATUS OF COLCHICINE-TREATED EWES WHICH RETURNED TO SERVICE

Day of colchicine treatment	Embryonic debris	Wt. of embryonic dry matter (g)	Ovarian status [follicle size (mm)]	Corpus luteum	
				Weight (g)	% type 4 lutein cells
13	Nil	<0·001*	9	0·1980	75·6
	Nil	<0·001	8	0·2210	81·9
	Nil	<0·001	R§	0·1105	68·7
	Nil	<0·001	10	0·2145	52·6
15	Nil	<0·001	7	0·1410	67·8
	Much†	0·019	N	0·2630	69·6
	Little‡	0·008	N	0·3230	57·2
	Little	0·006	N	0·1685	74·3
17	Nil	<0·001	8	0·0800	84·8
	Much	0·016	9	0·2940	68·6
	Nil	<0·001	7	0·2500	59·7
	Little	0·008	7	0·2505	66·0
19	Nil	<0·001	7	0·3875	77·1
	Little	0·006	7	0·2005	68·7
	Much	0·014	N	0·1040	82·5
	Much	0·021	9	0·1860	75·1

* Lower limit of sensitivity of technique.

† Many pieces of stringy debris suggestive of original membranes.

‡ Small sheets of cellular material.

§ R, recent ovulation; N, no large follicle.

(*d*) *Day 15 Colchicine Treatment*

Colchicine treatment on day 15 was followed by rapid degeneration and resorption of the embryo, though some residual debris remained *in utero* for up to 10 days. All six ewes autopsied on days 17, 19, and 21 after such treatment possessed debris *in utero*, though comparison of Tables 2 and 5(*a*) indicates that it was small in amount relative to the normal conceptus. The corpora lutea of these six ewes were comparable to those of normal pregnant animals in both weight and proportion of type 4 lutein cells.

Returns to service in the day 15 group occurred at times corresponding to cycle lengths of 23, 24, 26, and 28 days (mean 25·3±1·1). The ewe which returned to service on day 23 had no detectable debris *in utero* at autopsy, but the remaining three ewes contained varying amounts as detailed in Table 4. The corpora lutea of the four ewes which returned to service were small, pale, and contained a relatively high proportion of type 4 lutein cells. One of these ewes possessed a large pre-ovulatory follicle, but the other three had apparently quiescent ovaries (no follicles greater than 3 mm).

TABLE 5

CHARACTERISTICS OF THE EMBRYOS AND CORPORA LUTEA OF EWES AUTOPSIED AT INTERVALS AFTER COLCHICINE TREATMENT ON DAYS 15, 17, AND 19

Embryonic debris: S, small amount of debris present; M, moderate amount; L, large amount; L.P., large pieces; M.F., milky fluid. Membrane cell morphology: D, degenerate; P.N., pycnotic nuclei

Day of autopsy	Presence of embryonic debris*	Membrane length (cm)†	Embryonic dry matter (g)*	Membrane cell morphology*	Corpus luteum weight (g)†	Type 4 lutein cells (%)†
			(*a*) Colchicine treatment day 15			
17	S, S	—	<0·001,‡ 0·002	D, P.N.	0·7152	5·2
19	M.F., M	—	0·007, 0·011	D, D,	0·6165	0·9
21	S, M	—	0·009, 0·020	D, D	0·5252	13·3
			(*b*) Colchicine treatment day 17			
19	L.P., L.P.	25·7	0·014, 0·024	D, P.N.	0·5805	3·2
21	M, M	16·0	0·018, 0·028	D, D	0·6375	3·2
25	L.P., M	17·5	0·027, 0·019	D, D	0·8112	0·4
30	M, Nil	7·0	0·029, <0·001‡	D, D	0·4542	3·0
			(*c*) Colchicine treatment day 19			
21	L, L	31·7	0·040	P.N., P.N.	0·6080	1·5
25	M, L	14·2	0·060	P.N., D	0·6720	4·2
30	M, L	21·0	0·069	D, D	0·6970	1·1

* Individual data from two ewes on each day [except in (*c*) where mean of two observations are given for embryonic dry matter]. † Mean of two observations. ‡ Lower limit of sensitivity of technique.

(*e*) *Day 17 Colchicine Treatment*

The embryos of ewes treated with colchicine on day 17 degenerated rapidly, but Table 5(*b*) demonstrates that a considerable amount of debris remained in the uterus for at least a week after treatment. During this period the corpora lutea of affected ewes were normal in weight and lutein cell content. It was not until day 30 that there was any suggestion of complete embryonic resorption, though even then the corpora lutea were of normal lutein cell content and weight.

Day 17 ewes returning to service did so after cycle lengths of 33, 35, 36, and 37 days. Two of these four ewes had no detectable debris *in utero* at the time of autopsy, the remaining two had considerable debris (Table 4). All four ewes had a large pre-ovulatory follicle and a small, pale corpus luteum with a high proportion of type 4 lutein cells.

(*f*) *Day 19 Colchicine Treatment*

Comparison of the data in Tables 2 and 5(*c*) shows that the embryos of ewes treated with colchicine on day 19 degenerated rapidly in relation to the normal conceptus. It is also obvious, however, that a considerable amount of debris remained *in utero* until at least day 30 and that at that stage there were no signs of corpus luteum regression.

Ewes which returned to service did so after cycle lengths of 31, 38, 42, and 49 days, and three of the four ewes in this category had embryonic debris *in utero* (Table 4). At the time of return to service the corpora lutea of these four ewes were significantly reduced in weight and contained a large proportion of degenerating type 4 lutein cells.

IV. Discussion

Eight of the 16 colchicine-treated ewes which were allowed to return to service in the present study did so while appreciable quantities of embryonic debris remained *in utero*. Reference to Table 4 shows that these eight ewes were in the day 15, 17, and 19 treated groups; no day 13 ewes retained embryonic debris *in utero* until they returned to service. This difference is most likely related to the relatively small amount of embryonic material present on day 13. A noteworthy feature of the uteri of all ewes returning to service was the enlarged state of their caruncles relative to those of normal cyclic ewes returning to service. In the absence of satisfactory controls, no attempt was made to quantify this observation, but many of the caruncles were also slightly haemorrhagic and histological examination revealed that the surface epithelium of many of them was eroded, particularly in and adjacent to the body of the uterus. This markedly abnormal appearance of the uterus might be expected to be associated with either impaired sperm transport or survival or, if sustained, with failure of embryonic development in ewes which mate with fertile rams at this oestrus. With the small number of animals involved and the relatively infrequent checks for oestrus (twice daily), no particular significance can be placed on the fact that four of the eight ewes which returned to service with embryonic debris *in utero* had apparently quiescent ovaries.

The current findings confirm and extend the findings of Edey (1967) that ewes suffering early embryonic mortality may return to service while appreciable embryonic debris remains *in utero*. Edey (1970) has suggested that the fertility of ewes mated at the first oestrus after embryonic death may be depressed, and work is currently in progress in this laboratory to examine in greater detail the relationships between induced embryonic death, the presence of embryonic debris *in utero* at the subsequent oestrus, and sperm transport and fertility in ewes mated at this oestrus.

One problem encountered in this work was the quantification of embryonic debris *in utero*. The dry matter content of the uterine lumen was adopted as one such parameter, but examination of Tables 3–5 reveals that it is not entirely satisfactory. Interpretation of the dry matter data is complicated by differing rates of resorption, but in many cases it is obvious that the dry matter content of the uteri of ewes autopsied several days after colchicine treatment was substantially greater than that of the normal conceptus at the time of treatment. For example, the dry matter content of the normal day 19 uterus was 35–36 mg (Table 2). Examination

of Table 5 (*c*) shows, however, that ewes treated with colchicine on day 19 and autopsied as late as day 30 had up to three times this dry matter content. The macro- and microscopic evidence clearly indicates that colchicine treatment was followed by rapid embryonic death and degeneration, and that continued embryonic growth after colchicine treatment is thus unlikely to be the explanation for these apparently inconsistent dry matter results. Smears made to check on possible bacterial contamination revealed a marked leucocytic invasion of the uterine lumen in all ewes undergoing resorption, and histological examination revealed erosion of the surface epithelium of the caruncles. Both these effects could be expected to increase the dry matter content of the uterine lumen. Unfortunately, in the present study, it was not possible to accurately partition these effects nor to separate them from the contribution of the degenerating embryo and its membranes. Although leucocyte counts were not undertaken, the available smears suggested that the leucocyte content of the uterine lumen of ewes returning to service after embryonic death was considerably greater than normal. This factor might also be expected to play a detrimental role in the fertility of ewes mated at this oestrus.

The results of the present work indicate that embryonic degeneration and resorption following colchicine treatment between days 13 and 19 can be divided into two broad phases. In the first phase, of approximately 2 day's duration, rapid degeneration and disintegration of the embryo and its membranes occurs, with the macro- and microscopic integrity of both being quickly lost. Flat mounts of membrane fragments 2 days after colchicine treatment revealed cells which had either pycnotic nuclei or in which degeneration was so advanced that nuclei and cell boundaries could not be detected. During the second phase of degeneration and resorption, the duration of which varied between individuals and with time of embryonic death, gradual resorption of the embryonic debris occurred. Examination of Tables 3–5 indicates that in no case did corpus luteum regression (as assessed by a sharp increase in the proportion of type 4 lutein cells—Thwaites and Edey 1970) or return to service occur until virtually all embryonic debris had been removed from the uterus. In other words, the major reason for delayed return to service after embryonic death appears to be delayed embryonic resorption. All available evidence suggests that once most embryonic debris is removed from the uterus, corpus luteum regression proceeds rapidly and return to service occurs within a matter of days.

V. References

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