EMBRYO MORTALITY IN THE HEAT STRESSED EWE

I. THE INFLUENCE OF BREED

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Summary. In two separate experiments during the autumn of 1965 mature ewes of the Merino and Southdown breeds were continuously exposed to elevated temperatures in a hotroom for the first 20 days of pregnancy. Hotroom conditions in the two experiments were adjusted so as to stress similarly the ewes of both breeds. In both experiments hotroom treatment elevated rectal temperatures by approximately 2.5° F and respiratory rates by approximately 150 respirations/min. Such conditions led to the death of 100% of embryos in treated ewes. In both experiments approximately 75% of this embryonic death occurred at an early stage which did not interfere with subsequent returns to service after an oestrous cycle of normal length. The remaining 25% of the embryonic deaths occurred at later developmental stages and resulted in the presence of degenerating embryos *in utero* at autopsy on Day 23. It would appear that breed differences in heat-induced embryo mortality under similar environmental conditions are largely a consequence of breed differences in heat tolerance.

INTRODUCTION

The incidence of early embryo mortality is known to be significantly increased when ewes which are not acclimatized to heat are exposed to high ambient temperatures during early pregnancy (Dutt, Ellington & Carlton, 1959; Alliston, Egli & Ulberg, 1961; Alliston & Ulberg, 1961). It has been shown (Dutt, 1963) that under such conditions the incidence of embryonic mortality progressively increases to 100% as the interval from breeding to the beginning of exposure to heat decreases from 8 to 0 days. Little is known of factors which may modify the magnitude of this effect. Such factors could have implications in areas where sheep are mated under conditions which are conducive to heat stress. Fleece status had no significant effect on reproductive parameters in the hotroom experiments of Dutt *et al.* (1959). However, shorn ewes were consistently more tolerant of the experimental conditions than were unshorn ewes. The degree to which this superiority resulted from the superior heat tolerance of shorn sheep under hot/wet conditions (Lee, 1950; Klemm, 1962) is unknown.

The experiments described in this paper were undertaken to determine

whether the Australian Merino ewe is susceptible to this type of embryo mortality and whether a breed effect exists apart from the well-established breed differences in heat tolerance (reviewed by Hutchinson & Wodzicka-Tomaszewska, 1961).

MATERIALS AND METHODS

Experiment 1

Animals. Forty Merino ewes, aged 5 to 6 years, were shorn and placed in yards with a raddled (Radford, Watson & Wood, 1960) vasectomized ram on the 20th March 1965. The ewes ranged in weight from 34.9 to 48.9 kg (mean = 42.4 kg) and were subsequently maintained at this weight on a ration of equal parts sheep nuts and oaten chaff, the ration being fed communally at 15.00 hours daily.

Synchronization of oestrus. To facilitate subsequent experimental procedures it was desired to synchronize oestrus in the experimental animals. Each ewe was thus given a series of eight intramuscular injections of progesterone. The injections were given between 09.00 and 10.00 hours every 2nd day, beginning on 21st March, and each consisted of 20 mg progesterone in 2 ml peanut oil administered by automatic syringe in the gluteal region. Checks for ewes in oestrus during and after progesterone treatment were made twice daily, at 09.00 and 17.00 hours.

Fertile mating. In order to avoid any adverse influences of the exogenous progesterone, fertile mating was delayed until the second oestrus after progesterone treatment. This technique had the added advantage that a higher proportion of ewes could be expected to exhibit oestrus at the second cycle after progestagen treatment, due to a decline in the incidence of 'silent heat' (Edey & Thwaites, 1966). At 07.00 hours on Day 15 of the oestrous cycle after initial synchronization the ewes were placed indoors in well-lit and ventilated rooms with a fertile ram raddled with a fresh colour. From this time until the end of mating, oestrous checks were made at 12-hourly intervals, i.e. at 07.00 and 19.00 hours.

At each check ewes in oestrus were drafted off and hand-mated to two different fertile rams from a group held in reserve for this purpose. In addition, the fertile rams running with the ewes were regularly rotated at each check period, so that each ewe was served by at least three different rams.

Allocation to groups. Immediately following the oestrous check at which they would no longer stand for the ram the first thirty-two ewes were assigned either to a control or a hotroom group. The remaining eight ewes were discarded. The group size of sixteen was determined by the available hotroom space. Ewes within groups were considered as replicates and the ewes for each replicate were randomly assigned to groups. Control ewes were maintained indoors under natural temperature conditions (minimum temperature = 42° F, maximum temperature = 73° F) while the hotroom group was exposed continuously for 20 days to a dry-bulb temperature of $100 \pm 1^\circ$ F with 55 to 60% relative humidity.

Determination of ovulation rate. On the 4th day after fertile mating all ewes

underwent midventral observational laparotomy (Lamond & Urquhart, 1961) and their corpora lutea were counted.

Returns to service. Checks for ewes returning to service were made twice daily from the time of allocation to groups until Day 23, raddled vasectomized rams being run with the ewes for 30 min night and morning for this purpose. On Day 23 after fertile mating those ewes which had returned to service were again laparotomized to confirm that ovulation had again taken place.

Post-experimental period. The elevated water intake of the hotroom group (see 'Results') made it impossible to obtain a liveweight recording comparable to that in controls when the ewes were removed from the hotroom at the end of Day 20. After removal from the hotroom, ewes of the treated group were thus placed in conditions similar to those experienced by controls and were weighed daily for 3 days. By that stage excess fluid had apparently been excreted, for liveweight had levelled off.

Slaughter and embryo recovery. All ewes which had not returned to oestrus by the afternoon of Day 23 were immediately slaughtered. The uteri were removed and placed in warm saline for transport to the laboratory. Within 10 min of slaughter the uteri were opened and any embryos or embryonic remains present were removed, examined under a binocular microscope, classified as normal or abnormal and photographed. The criteria used for classifications of embryos were: vascularity of the embryo and allantois, heart development and size of embryo and allantois.

Indices of heat stress. Rectal temperatures (by clinical thermometer inserted 4 in.) and respiration rates (average of two $\frac{1}{2}$ -min flank movement counts) were recorded at 14.00 hours on the day before and on Days 1, 10 and 20 during the experimental period, as a measure of the stress imposed by the hotroom treatment. In addition, records were kept of the daily total feed and water intakes of both groups.

Experiment 2

On 26th April 1965, forty Southdown ewes, aged 5 to 6 years and weighing from 29.0 to 56.6 kg (mean = 40.9 kg) were shorn and placed in yards. These ewes were thereafter subjected to the same experimental procedures as have been outlined above for the Merino ewes of Exp. 1. The two experiments differed in only two details. Due to seasonal temperature changes the control ewes of Exp. 2 experienced slightly lower temperatures (minimum temperature = 38° F, maximum temperature = 66° F) than did those of Exp. 1. Southdowns are known to be less tolerant of heat than Merinos (Miller & Monge, 1946) and thus in Exp. 2 the hotroom was maintained at $95 \pm 1^\circ$ F dry-bulb temperature and 55 to 60% relative humidity; conditions which preliminary trials showed would stress the Southdown ewes to a similar degree as the conditions in Exp. 1 stressed the Merino ewes.

RESULTS

Synchronization of oestrus

In Exp. 1 82.5% of ewes and 80.0% of those in Exp. 2 were in oestrus within

the period from 3 to 6 days after the final progesterone injection. Corresponding figures for ewes in oestrus at the second cycle were 85.0 and 89.5% respectively.

Rectal temperature and respiration rate

Rectal temperatures and respiration rates of control ewes in both experiments were very similar, mean values at the different recording times varying between 101.49 to 101.96° F and 15.25 to 20.50 respirations/min respectively. Table 1 illustrates the significant increase which occurred for both of these parameters during hotroom treatment. In both experiments rectal temperature and respiration rate in treated ewes rose to approximately 104.4° F and 170 respirations/min respectively 24 hr after the beginning of hotroom exposure. Thereafter

TABLE 1
RECTAL TEMPERATURES AND RESPIRATION RATES OF CONTROL AND HOT-ROOM EWES

Days after oestrus	Experiment No. 1		Experiment No. 2	
	Control	Hotroom	Control	Hotroom
0	101.80 ± 0.15‡ 20.50 ± 0.82§	101.80 ± 0.16 19.00 ± 0.77	101.58 ± 0.22 16.00 ± 0.56	101.89 ± 0.15 17.75 ± 0.40
1	101.96 ± 0.13 17.75 ± 0.50¶	104.46 ± 0.20*† 169.62 ± 3.55*†	101.62 ± 0.23 16.06 ± 0.55	104.29 ± 0.18*† 171.62 ± 2.45*†
10	101.76 ± 0.13 18.00 ± 0.47	104.28 ± 0.14* 163.12 ± 3.02*	101.64 ± 0.13 16.12 ± 0.67	104.26 ± 0.17* 150.37 ± 3.62*
20	101.59 ± 0.14 15.87 ± 0.60¶	103.81 ± 0.14*¶ 148.87 ± 3.55*	101.49 ± 0.13 15.25 ± 0.58	103.52 ± 0.14* 129.37 ± 6.20*¶

‡ Rectal temperature (°F).

§ Respirations/min.

* Significantly greater than corresponding control value, $P < 0.001$.

† Significantly greater than preceding value, $P < 0.001$.

¶ Significantly less than preceding value, $P < 0.050$.

|| Significantly less than preceding value, $P < 0.010$.

both parameters declined slowly until Day 20, at which stage they remained significantly higher than the corresponding control values.

Ovulation rate

Table 2 summarizes the results of the corpora lutea counts which were made in all ewes after laparotomy on Day 4. It can be seen that although ewes were allocated to their groups before laparotomy, differences between groups in ovulation rate and the occurrence of twin ovulations were small.

Fertilization rate

Due to the restricted group size it was not possible to assess fertilization rates directly, by slaughter and ovum recovery, in these experiments. It is, thus, not possible to separate fertilization failure from early embryonic deaths which resulted in returns to service after a cycle of normal length. However, since treatment was delayed until after the estimated time of fertilization, it may be

TABLE 2
DISTRIBUTION OF CORPORA LUTEA IN EWES OF THE CONTROL
AND HOTROOM GROUPS OF EXPERIMENTS 1 AND 2

	Experiment No. 1		Experiment No. 2	
	Control	Hotroom	Control	Hotroom
No. of ewes	16	16	16	16
Single corpora lutea				
Right ovary	5	5	5	5
Left ovary	6	5	5	7
Sub-total	11	10	10	12
Twin corpora lutea				
Right ovary	0	6	4	0
Left ovary	8	0	4	2
Bilateral	2	6	4	6
Sub-total	10	12	12	8
Total corpora lutea	21 (1.31)*	22 (1.37)	22 (1.37)	20 (1.25)

* Ovulation rate (corpora lutea/ewe).

assumed that fertilization rates were similar in all groups. Twenty-one-day non-return rates in the control groups of Exps. 1 and 2 were 75.0 and 81.25% respectively; these values give a very conservative estimate of fertilization rate.

Returns to service and embryo mortality

Five (31.25%) control ewes in Exp. 1 returned to service before Day 23, as compared to 12 (75.0%) ewes in the corresponding hotroom group. Tested by χ^2 this difference proved to be significant at the 0.2% level. Table 3 shows that a similar trend was also present in Exp. 2, although in this case the difference between groups just failed to reach significance ($\chi^2 = 3.137$; $0.05 < P < 0.10$).

TABLE 3
RETURNS TO SERVICE AND EMBRYONIC MORTALITY IN THE EWES OF
EXPERIMENTS 1 AND 2

	Experiment No. 1		Experiment No. 2	
	Control	Hotroom	Control	Hotroom
No. of ewes	16	16	16	16
Returns to service				
No. of ewes	5	12*	6	11
% ewes	31.25	75.00	37.50	68.75
No. of ova shed	21	22	22	20
Ova not represented by viable 23-day embryos				
No.	6	22†	8	20†
%	28.57	100.00	36.36	100.00

* Significantly more than in control group, $0.01 < P < 0.02$.

† Significantly more than in control group, $P < 0.001$.

In both experiments none of the ova shed by ewes in the hotroom groups were represented by viable embryos at autopsy on Day 23. In the corresponding control groups 71.43 and 63.64% of ova shed were represented by viable embryos at this stage (Table 3). In both experiments this difference in embryo mortality rate was highly significant ($P < 0.001$).

Feed and water intake

Throughout both experiments all ewes were communally fed, in groups, a daily ration consisting of 0.75 lb/head of both sheep nuts and oaten chaff. In the control groups this ration was always completely consumed and was sufficient to maintain group mean liveweights at a constant level. The oaten chaff portion of the ration was always completely consumed by both hotroom groups although their consumption of nuts declined to approximately 50 to 60% of that in controls by about the 5th day after exposure to heat. Thereafter the consumption of nuts by both hotroom groups progressively increased until approximately Day 10, at which stage complete consumption of the ration was resumed.

Drinking water, at ambient temperature, was freely available at all times to all ewes. Control ewes in Exps. 1 and 2 consumed an average of 1.17 ± 0.11 and 1.52 ± 0.32 l. of water/ewe/24 hr, respectively. Hotroom exposure led to an immediate increase in water intake, in Exp. 1 to an average of 6.86 ± 0.55 and in Exp. 2 to an average of 7.74 ± 0.82 l./ewe/24 hr. Differences between groups in both experiments were highly significant ($P < 0.001$). In both experiments the liveweight of ewes showed an apparent increase of approximately 2.50 kg during hotroom exposure. This weight gain was lost during the 1st day after hotroom treatment and was apparently a consequence of the elevated water intakes of the hotroom groups.

DISCUSSION

Ovulation rate

Although the ewes were allocated to groups before laparotomy, the ovulation rate and occurrence of twin ovulations was similar in all groups. This similarity can be attributed, at least in part, to the similarity in group mean liveweights (Coop, 1962). Of the eighty-five ovulations observed in these experiments forty-six (54.1%) occurred in the left ovary and thirty-nine (45.9%) in the right. This distribution is in agreement with the recent report of Edey (1966). Previous studies of abattoir material (Henning, 1939; Hunter, 1959), super-ovulated ewes (Pursel & Graham, 1962) and of ewes at laparotomy (McKenzie & Terrill, 1937) have indicated greater ovulatory activity in the right ovary of the ewe.

Rectal temperature and respiration rate

Values for these parameters in the control Merino and Southdown ewes of Exps. 1 and 2 respectively varied little from the overall means of 101.68°F and 16.94 respirations/min. The available literature suggests that recordings of this magnitude might be expected 24 hr after feeding newly-shorn ewes which are housed indoors under cool/temperate conditions. The slight, but significant,

decline in the respiration rate of the control ewes of Exp. 1 is not related to any general decline in the ambient temperatures experienced by them. Although accustomed to regular handling and the experimental conditions, the Merino ewes of Exp. 1 were not, at the start of the experiment, as tractable as were the Southdown ewes of Exp. 2 at a similar stage. It seems likely that the gradual decline in the respiration rate of the control Merino ewes is related to their increasing tractability as the experiment progressed.

Table 1 amply illustrates the highly significant increases in rectal temperature and respiratory rate which were induced by hotroom treatment in both experiments. The gradual decline in both these parameters over the 20-day hotroom exposure is presumably an indication of a gradual acclimatization to the high temperatures, although at the end of treatment both values for hotroom ewes remained significantly above those for their corresponding controls.

Southdown ewes have been observed to be less tolerant of heat than Merinos under field conditions (Miller & Monge, 1946) and in the climate chamber (unpublished observations from this laboratory). This breed difference in heat tolerance is borne out in the present experiments, for hotroom treatment led to a similar rise in rectal temperature and respiration rate in both breeds although the hotroom was maintained 5° F cooler for the Southdowns than for the Merinos.

Embryonic mortality

Of the ova shed by ewes of the control groups of Exps. 1 and 2, 28.57 and 36.36% respectively were not represented by viable embryos at autopsy on the 23rd day of pregnancy. As embryonic loss after 3 or 4 weeks of gestation in the sheep is relatively rare (Robinson, 1957; Foote, Pope, Chapman & Casida, 1959) an embryonic death rate of this magnitude in untreated ewes is within the range indicated by most previous reports (reviewed by Edey, 1966), a range which may be considered, at least in occurrence, to be 'normal'.

In neither experiment were the ova shed by ewes in the hotroom groups represented by viable 23-day embryos. Exposure of ewes to high ambient temperatures at various stages of the reproductive cycle has been shown to have a detrimental effect on fertilization rate (Dutt *et al.*, 1959; Alliston & Ulberg, 1961) and embryo survival (Dutt *et al.*, 1959; Alliston *et al.*, 1961; Dutt, 1963), although the unfertilized ovum is apparently not affected (Woody & Ulberg, 1964). In the present experiments it was desired to restrict the influence of high temperature to the period of the developing embryo and thus ewes were placed in the hotroom at the oestrous check at which they would no longer stand for the ram. Since checks were at 12-hourly intervals ewes entered the hotroom an average of 6 hr after the end of oestrus.

Ewes have been shown to ovulate towards the end of oestrus (Green & Winters, 1935; McKenzie & Terrill, 1937; Woody & Ulberg, 1964) so that fertilization in the ewes in these experiments should have been completed by, or soon after, the time of entry into the hotroom. Dutt (1963) has shown, furthermore, that the fertilization rate is not significantly affected when ewes are suddenly exposed to high temperatures on the day of oestrus. It can thus be confidently assumed that the effects of the high temperature treatments

employed in these experiments were confined to the period of the segmenting ovum and the developing embryo.

The observed embryo mortality rate of 100% in both the present experiments is in agreement with the results of Dutt (1963). This worker has shown that the embryo mortality which results from hotroom exposure of heat-unacclimatized ewes progressively declines from 100% as the interval from mating to the beginning of treatment increases from 0 to 8 days. Eighteen of the twenty ewes placed in the hotroom by Dutt (1963) on either the day of breeding or the day thereafter returned to oestrus after 15 to 19 days; the remaining two ewes, which were from the group placed in the hotroom on the day after breeding, subsequently lambed. Since the presence, *in utero*, of a normal-sized embryo may be necessary on Day 12 or 13 to prevent regression of the corpus luteum (Moor & Rowson, 1964, 1966) it would appear that embryonic death in these eighteen ewes occurred before the 10th day after mating. Studies under similar conditions and involving ovum recovery (Dutt, 1963) and embryo transfer (Alliston & Ulberg, 1961) techniques indicate that this embryonic death may occur while the ova are undergoing cleavage in the Fallopian tubes; i.e. as early as Day 3.

In the current experiments twenty-three of thirty-two (71.9%) treated ewes returned to oestrus within 13 to 23 days after mating. Neither the number of ewes returning nor the mean length of the post-breeding oestrous cycles (overall average = 17.6 days) differed significantly between experiments. The uteri of the remaining nine (28.1%) treated ewes (four from Exp. 1; five from Exp. 2) contained embryos which were judged to be non-viable at autopsy on Day 23. These represented a range of degenerate types; from the subnormally-sized embryo, showing little gross degeneration but having poor vascularity and no heartbeat, through to the almost completely degenerated type consisting of macroscopically unrecognizable debris.

Plate 1 contrasts the normal 23-day embryo (Pl. 1, Fig. 1) with representatives of the range of embryonic degeneration observed in the above non-viable group (Pl. 1, Figs. 2 to 4). Study of these embryos suggested that oestrus may have recurred within days in ewes containing embryos in advanced stages of degeneration. It seemed equally likely, however, that it may have been delayed for weeks in those ewes which carried embryos which were dead but which were also developmentally well advanced and showing little sign of gross degeneration. Lack of information on the time course of embryonic degeneration in the ewe and of the recurrence of oestrus thereafter makes it impossible to be at all precise in these estimations.

Delayed embryonic death of this type in ewes subjected to heat stress immediately after oestrus has not previously been reported. The hotroom treatment employed in the present experiments increased rectal temperatures by approximately 2.5° F and respiratory rates by approximately 150/min. In the experiments of Dutt (1963), in which no interference with the cyclical oestrous rhythm was observed in ewes treated at a similar time, hotroom exposure elevated rectal temperatures by an average of 3.2° F. It is thus possible that these observed differences are related to the different degrees of stress imposed in the various experiments. Previous results (Dutt *et al.*, 1959) from the hotroom

The normal 23-day sheep embryo and the various degrees of embryonic degeneration, observed in hotroom ewes at autopsy on Day 23. All 1963.

FIG. 1. Normal 23-day sheep embryo.

FIG. 2. Embryonic remains in advanced stage of degeneration.

FIG. 3. Twin embryos in an intermediate stage of degeneration.

FIG. 4. Dead embryo, sub-normal in size but showing little gross degeneration.

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exposure of shorn and unshorn ewes indicate that embryonic death rates increase with increasing degrees of heat stress. It is also possible that these differences stem from an age-of-ewe effect, for Shelton (1964) has observed that young ewes are more seriously affected by summer climatic stresses than are older ewes.

The present results correspond more closely, in this regard, to those reported by Dutt (1963) for groups which were subjected to more stressful hotroom conditions beginning on Days 3 and 5 after mating. In these groups four ewes (10.0%) returned to service from forty-one to sixty-six post-breeding and seven ewes (17.5%) did not return to service but did not lamb. This interference with the cyclical oestrous rhythm is presumably due to delayed embryonic death and the presence of degenerating embryos *in utero*.

Breed differences in heat tolerance, although somewhat indefinite, are well-known (Hutchinson & Wodzicka-Tomaszewska, 1961). Thus, under similar environmental conditions, breed differences in heat-induced embryo mortality might be expected. The fact that, in the work reported here, similarly heat stressed Merino and Southdown ewes suffered very similar embryo mortality strongly suggests that such differences are primarily a consequence of the breed differences in heat tolerance. In view of the delayed embryonic death which occurred in 28.1% of the treated ewes in these experiments the observed response cannot be considered maximal, even though the rate of embryonic death was 100%. The results do not provide any information concerning possible breed differences in the relationship between degree of heat stress and embryo mortality. There is no reason to believe, however, that such differences would be of any importance.

The contrast between the results of the present work and that of Ryle (1961) emphasizes the importance of acclimatization to heat and degree of heat stress as factors modifying the rate of embryo mortality in the heat stressed ewe.

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