

## Detrimental Effects of High Ambient Temperature on Fertility and Early Embryo Survival in Sheep

by  
R. H. Dutt\*\*

The climatic environment influences nearly every aspect of plant and animal life including that of reproduction. It is becoming clear that there are certain optimal environmental conditions which are essential to maximum reproductive performance in many species of animals. Although there is little doubt that season affects the reproductive efficiency of animals, there are few quantitative data on the specific effects of high ambient temperature in this regard. Recent investigations of the causes of reproductive failure in sheep early in the breeding season suggest that one factor is lowered fertility of rams. Fertility is improved in ewes early in the season if rams are kept at cooler temperatures during the summer months; however, embryo loss is abnormally high (Dutt and Simpson, 1957).

The frequent occurrence of morphologically abnormal ova early in the breeding season reported by Dutt (1954) and Hulet et al. (1956) and the reported estimates of high embryo mortality lead one to suspect that certain factors causing lowered fertility may be associated with the female reproductive system.

The present study was designed to investigate the effects of high ambient temperature on the breeding performance of ewes. Ewes were exposed to the high temperature before, during and at precise intervals after breeding, and the effects on the estrual cycle, fertility rate, morphology of ova and embryo loss were determined.

### MATERIALS AND METHODS

The treated ewes were placed in an insulated room in which the temperature was maintained constantly at 32°C, with a variation of not more than 1°C, and with the relative humidity maintained at 60-65%. Previously these environmental conditions were found to impair spermatogenesis in the ram. Two sides of the room contained unshaded thermopane windows which permitted approximately normal light conditions. Forced ventilation was provided so that stagnant air and ammonia odor were eliminated.

Control ewes were kept in an open-sided barn with an outside dry lot. Nutrition was adequate and similar for all ewes. The study was conducted during four breeding seasons and was planned so that breeding occurred during November, when the mean daily outside temperature ranged from -6°C to 20°C. A total of 200 crossbred (grade Rambouillet ewes x mutton ram) ewes were included in the study. All ewes were checked for estrus daily in the morning with aproned teaser rams and were considered to be in estrus if they stood and allowed a ram to mount. In order to minimize variation in fertility due to rams, ewes were artificially inseminated with 0.5 ml of fresh, undiluted semen pooled from at least three fertile rams. Ewes were bred one time, between 15:00 and 17:00 hr on the first day of estrus.

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\*\*\*) Kentucky Agricultural Experiment Station, University of Kentucky, Lexington, Kentucky, U.S.A.

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One group of ewes was placed in the heated room on the twelfth day of the cycle (before-breeding group) and the ewes were bred at the subsequent estrous period. A second group was bred and immediately placed in the heated room (0-day group), and another group of ewes was placed in the heated room 1 day after breeding (1-day group). Three additional groups of ewes were bred and placed in the heated room 3 days after breeding (3-day group), 5 days after breeding (5-day group), and 8 days after breeding (8-day group), respectively. This design permitted a study of the effect of high temperature on fertility as well as early embryo mortality prior to and after the embryo enters the uterus. All unslaughtered treated ewes were kept in the heated room until they returned to estrus or until 24 days had passed after breeding, at which time they were removed and placed with the control ewes. Ewes retained for lambing were checked for estrus daily with an aproned teaser ram for at least 50 days after breeding, in order to determine whether the treatment affected the incidence of subsequent estrus and the normal cyclic pattern.

Fertility rate was determined by slaughtering half of the ewes in the control, 0- and 1-day groups 3 days after breeding and examining the reproductive tracts in the laboratory. The number of corpora lutea were recorded, and the oviducts were dissected from the mesosalpinx and severed at the tubo-uterine junction. Ova were recovered by flushing the oviducts and uterine horns with 0.85% saline solution. The flushing fluid was collected in watch glasses, and after the ova were located with a dissecting microscope, they were transferred to a hanging drop slide and examined under high power (430 x) for cleavage and evidence of morphological abnormalities. The number of sperm cells present in the zona pellucida in a medium plane of the ovum were also recorded. Data from control ewes were used as an estimate for fertility in the 3-, 5- and 8-day groups, since fertilization would already have been completed before these groups were exposed to the high temperature.

Embryonic mortality was calculated using fertility data for the slaughtered ewes and lambing data for the remaining ewes which were allowed to go to term. Rectal temperatures were taken with a thermistor thermometer on random samples of ewes from the treated groups as an indication of thermal stress.

The  $X^2$  test corrected for continuity was used for statistical analysis of the data on reproductive performance (Steel and Torrie, 1960). The temperature and estrous cycle data were tested for significance by analysis of variance.

## RESULTS

**FERTILITY.** Fertility rate is given in Table 1. Fertility of the control ewes was consistently high during the four years of the study. This is probably due to restricting breeding to the period of the year when fertility is normally high and to the use of pooled semen from several rams.

TABLE 1. Ovulation and fertility rates and percent abnormal ova in ewes exposed to 32°C ambient temperature before, at and after breeding

Ewe treatment group	Number of ewes	Number of corpora lutea	Number of ova recovered	Percent of ova fertilized	Percent abnormal ova*
Control	40	56	52	94.2	3.8
Before breeding	20	27	27	40.7***	55.6***
0-day	10	13	13	69.2**	46.2***
1-day	10	13	13	100.0	30.8**

\*) Includes both unfertilized and fertilized ova.

\*\*\*) Significantly different from control ewes,  $P < .05$ .

\*\*\*\*) Significantly different from control ewes,  $P < .01$ .

Percent of ova cleaved was significantly lower ( $P < 0.1$ ) in the ewes exposed to the high temperature on the twelfth day of the cycle (40.7% vs. 94.2% for the controls). Fertility was also lower ( $P < 0.5$ ) for the 0-day group. Ewes in this group were exposed to the high temperature at time of breeding, and fertilization would have occurred about 24 hr later. Rectal temperatures of the ewes in this group averaged  $39.1^{\circ}\text{C}$  just before they were placed in the heated room and  $40.8^{\circ}\text{C}$  24 hr later, a highly significant difference.

In the 1-day ewes fertility was not affected by the high temperature. The interval between estrual checks was 24 hr, and these ewes would have been exposed to heat an average of 44 hr (one-half the interval between estrous checks plus 32 hr) after onset of estrus. This interval is slightly longer than the average duration of estrus for similar ewes reported by Dutt, Ellington and Carlton (1959). Since ovulation is reported to occur near the end of estrus (McKenzie and Terrill, 1937), ewes in this group should have ovulated, and fertilization would have been effected before or shortly after exposure to heat (Green and Winters, 1945).

Ovulation rate in the ewes that were subjected to high temperature before breeding was not affected (Table 1). The length of the estrous cycle for ewes exposed to the high temperature before breeding was significantly ( $P < 0.05$ ) longer than for the control ewes (16.8 days vs. 16.2 days). However, the high temperature did not interfere with subsequent estrus, and all of the treated ewes came into estrus within the expected time interval. Duration of estrus was not significantly different for the control and treated ewes (1.9 days vs. 1.6 days, respectively).

**ABNORMAL OVA.** The summarized data for abnormal ova include both unfertilized and fertilized ova. The percent of abnormal ova in the ewes exposed to heat before breeding and in the 0-day group was significantly ( $P < 0.01$ ) higher than for control ewes (Table 1). In these two groups 55.6% and 46.2%, respectively, of the ova were classified as abnormal. In the 1-day group all of the ova were cleaved, but 30.8% ( $P < 0.05$ ) were classified as abnormal (vacuolated or disintegrating cells). Photomicrographs of various types of ova abnormalities observed are shown in Fig. 1. The 4-celled ovum (D) is definitely not normal. The vacuoles in the cytoplasm are believed to be the result of degenerative processes and an indication that the embryo is dead. Hart (1956) has reported finding abnormal fertilized ova in sheep, but his data offer no estimation of subsequent embryo loss.

**LAMBING PERCENT.** Percent of ewes lambing was lower in the ewes exposed to the elevated temperature (Table 2). Of the 40 control ewes which were retained to term, 87.5% lambed. None of the ewes exposed to high temperature before breeding or at the time of breeding lambed. Twenty percent of the ewes in the 1-day group, 35% in the 3-day group, 40% in the 5-day group and 70% in the 8-day group lambed. Percent of ewes lambing was significantly ( $P < 0.01$ ) lower in all of the treated groups except the 8-day group. Lambing percent in this group was lower than for the control ewes, but the difference was not significant.

TABLE 2. Percent of ewes lambing after exposure to  $32^{\circ}\text{C}$  ambient temperature before breeding, at time of breeding and at various intervals after breeding

Ewe treatment group	Number of ewes	Percent of ewes lambing	Number of lambs born	Lambing rate
Control	40	87.5	43	1.2
Before breeding	10	0.0*	--	--
0-day	10	0.0*	--	--
1-day	10	20.0*	2	1.0
3-day	20	35.0*	10	1.4
5-day	20	40.0*	9	1.2
8-day	10	70.0	8	1.1

\*) Significantly lower than control ewes,  $P < 0.01$ .

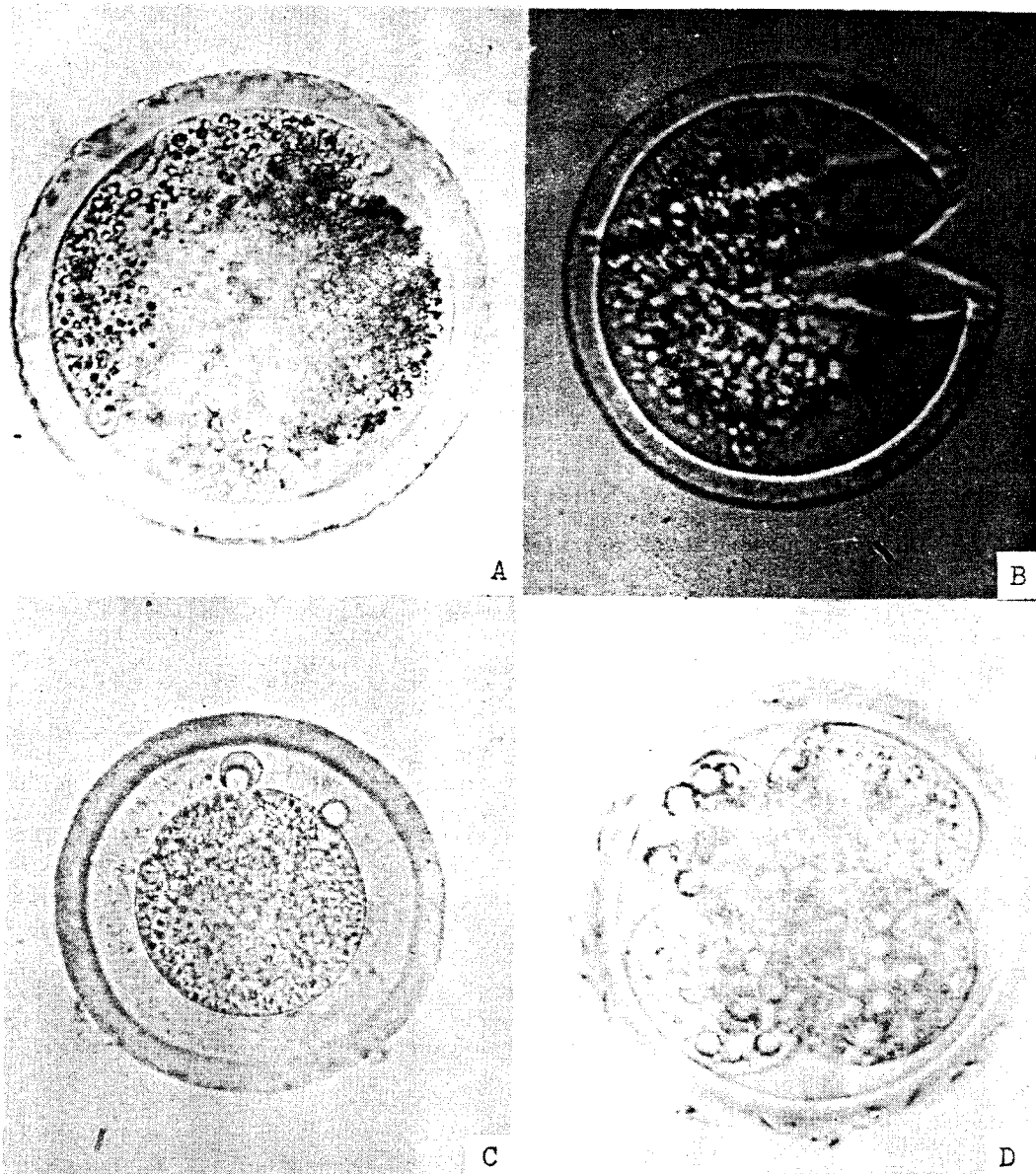


Fig. 1. Photomicrographs of morphologically abnormal ova recovered from ewes after exposure to 32°C ambient temperature. All taken at 430 magnification.

- A. Ovum showing ruptured vitelline membrane.
- B. Ovum with the zona pellucida broken.
- C. Ovum with cytoplasm shrunken and vacuoles present in the perivitelline space. A, B and C were recovered from ewes placed in the heated room before breeding.
- D. Cleaved four-celled ovum recovered from a 1-day ewe. Vacuoles in the cytoplasm are believed to indicate degenerative processes and that the embryo is dead. Sperm cells can be readily identified in the zona pellucida.

In the 3-, 5- and 8-day groups the lower lambing percent resulted entirely from increased embryo mortality, since ewes in these groups were not exposed to the heat until after fertilization had been completed. In the remaining treated groups lowered fertility was also responsible for the decrease in lambing percent, since these ewes were exposed to the elevated temperature before fertilization had occurred.

**ESTIMATED EMBRYO MORTALITY.** Embryo mortality was calculated using fertility data from the slaughtered ewes and the lambing rate from corresponding groups of ewes allowed to go to term. Estimates of embryo mortality are given in Table 3 and represented graphically in Fig. 2. In the control ewes 12.2% of the embryos were lost between 3 days post-breeding and lambing. All of the embryos died in the ewes exposed to heat before breeding and in the 0-day group. Embryo mortality in the 8-day ewes was higher (38.5%) than in the control ewes, but the difference was not significant. For the remaining treated groups embryo mortality was significantly ( $P < 0.01$ ) higher than in the control ewes.

TABLE 3. Estimated embryo mortality in ewes exposed to 32°C ambient temperature before breeding, at time of breeding and at various intervals after breeding \*)

Ewe treatment group	Number of ova fertilized in slaughtered ewes	Number of lambs born by term ewes	Estimated embryo mortality, %
Control	49 (40)	43 (40)	12.2
Before breeding	11 (20)	0 (10)	100.0**
0-day	7 (10)	0 (10)	100.0**
1-day	9 (10)	2 (10)	77.8**
3-day	26 (20)	10 (20)	61.5**
5-day	26 (20)	9 (20)	65.4**
8-day	13 (10)	8 (10)	38.5

\*) Calculated according to the following formula:

$$\frac{\text{fertilized ova minus lambs born}}{\text{fertilized ova}} \times 100.$$

Figures in parentheses represent the number of ewes.

In the 3-, 5- and 8-day groups fertilization would have occurred before the ewes were exposed to the high temperature, and therefore the number of fertilized ova from control ewes bred contemporaneously with these three groups was used in calculating embryo mortality. Thus the ewes shown for fertility data for these groups are also included in the control group.

\*\* ) Significantly higher than control ewes,  $P < 0.01$ .

When the data for the before-breeding, 0- and 1-day groups were combined, embryo mortality was significantly ( $P < 0.01$ ) higher than for the ewes in the combined 3-, 5- and 8-day groups. The sheep ovum enters the uterus during the third or fourth day after estrus, and the 3-day group was exposed to the high temperature just before the ova would normally be leaving the oviduct and passing into the uterus. These results demonstrate that the young sheep embryo is less sensitive to environmental heat stress and has a better chance of surviving after entering the uterus. Furthermore, ewes in all treated groups, except the 8-day ewes, were subjected to the elevated temperature environment before the embryos had reached the blastocyst stage (Green and Winters, 1945). The non-significant difference in embryo mortality between the 8-day group and control ewes demonstrates that the embryo is less sensitive to the effects of unfavourable maternal environment after it develops into a blastula. The

differences in embryo mortality between the 5- and 8-day groups (ewes in both groups not exposed to the high temperature until after the embryos were in the uterus) support this observation.

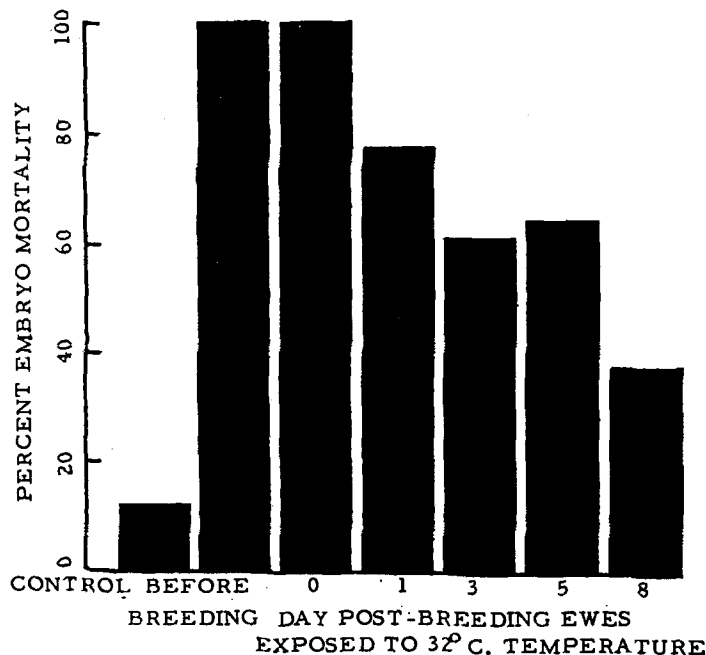


Fig. 2. Estimated embryo mortality in control ewes and in ewes exposed to 32°C ambient temperature on the twelfth day of the cycle (before breeding), at time of breeding (0-day) and 1, 3, 5, and 8 days after breeding.

#### DISCUSSION

Under conditions of high environmental temperature or high humidity, when an increase in body temperature of the female results, there exists the possibility of (1) cessation of estrous activity or ovulation; (2) a lower fertilization rate, or (3) an increase in embryonic death loss. Twenty-seven of the thirty ewes in the before-breeding, 0- and 1-day groups returned to estrus within 15 to 19 days after breeding. This interval is considered to be within the normal range for the estrous cycle of the ewe. Ewes in these groups were in the heated room for the entire interestrous period, and the cyclic rhythm was not significantly altered by the temperature stress, except possibly in one ewe that returned to estrus 34 days following breeding. There is no evidence that estrous activity or ovulation rate was affected by subjecting ewes to the elevated temperature before breeding.

In the 3-, 5-, and 8-day groups 28 of the 50 ewes failed to lamb. Fifteen of these returned to estrus within 14 to 20 days after breeding, 4 at intervals ranging from 41 to 66 days, and 9 ewes that did not lamb failed to return to estrus. The interference of the normal cyclic pattern in the ewes of these 3 treated groups is apparently due to the presence of degenerating embryos. The basis for this conclusion is the fact that there was no interference of the cyclic pattern in ewes exposed to the elevated temperature before the embryo entered the uterus (before breeding, 0- and 1-day groups).

It is possible that the treatment may have affected rate of ovum travel through the oviduct. In the control ewes 9.6% of the ova were recovered from the uterine horns, and in the before-breeding, 0- and 1-day groups 11.3% of the ova were recovered

from the uterine horns. The remaining ova were recovered from the oviducts. Thus, there is no evidence that rate of travel of ova through the oviduct had been speeded up. Ewes were not slaughtered later than 3 days after breeding, and critical information on whether there was any retardation in rate of travel is not available. However, the similarity between the percent of ova recovered from the uterus in the control and treated ewes suggests that there was no retardation.

The marked decrease in fertilization rate may indicate that sperm livability or travel was impaired. Fewer recovered ova from ewes of the before-breeding group contained sperm cells in the zona pellucida (15.5% compared with 88.9% for the control ewes). This fact suggests that sperm may have been adversely affected while in the reproductive tract of the female. However, the higher percent of abnormal ova in the treated ewes raises the question whether failure of fertilization was due to inability of the sperm cells to reach the ova or on their inability to penetrate initially defective ova. It is also probable that increased body temperature (average 1.7°C) in ewes exposed to the heat had a damaging effect *per se* upon the gametes. Dutt, Ellington and Carlton (1959) found that shearing ewes before exposing them to high environmental temperature tended to lessen the detrimental effects upon fertility and embryo mortality (fleece weights ranged from 5 to 7 lb).

The fact that fertility is decreased and the incidence of morphologically abnormal ova is increased in ewes exposed to elevated ambient temperature before breeding is of considerable interest in view of existing seasonal differences in fertility. Stott and Williams (1962) concluded that a lower fertilization rate and high embryonic mortality are important factors in lowered breeding efficiency in dairy cows bred during periods of high temperatures and humidity. These findings raise the question as to whether some of the seasonal differences in fertility reported for livestock might be due in part to changes in the physiological environment within the female reproductive system rather than entirely to changes in fertility level of the male (Dutt, 1954; Hilder, Fohrman and Groves, 1944; Mercier and Salisbury, 1946; Hulet et al., 1956).

Exposing females to elevated temperature has been shown to be harmful for successful reproduction in certain species. Yeates (1953) has reported an adverse effect of high temperature during gestation in ewes. Similarly, Sundstroem (1927) in studies with rats and Ogle (1934) with mice reported hot-room treatment resulted in small litters.

Data from the present and other studies (Shah, 1956; Aldred, Stob and Andrews, 1961; Alliston and Ulberg, 1961; Alliston, Egli and Ulberg, 1961) suggest high ambient temperature as a possible cause of poor conception in ewes when bred early in the season, as reported by Dutt (1954) and Hulet et al. (1956). These studies offer unquestionable support for the conclusion that thermal stress can reduce fertility and result in an increase in embryo loss in ewes. Furthermore, the data show that the embryo is most susceptible to damage while it is in the oviduct; however, this does not imply that embryo loss cannot occur at later stages of pregnancy. When the harmful effects of elevated environmental temperature upon reproductive performance in ewes are partitioned, they appear to be far greater upon fertilization rate and survival of the embryo prior to and during the early blastocyst stage than upon estrual rhythm, ovulation rate or embryo survival at later stages in pregnancy.

Ewes exposed to the high temperature showed a significant increase in body temperature within 24 hours. The harmful effects from the temperature stress could result from (1) the direct effect of a rise in body temperature, (2) biochemical effects such as a change in enzyme activity or metabolic function, or (3) a disturbance in endocrine balance. There was no difference in the appearance of the uterine endometrium in histological sections prepared from control and treated ewes. If there is any change in the pituitary-ovarian balance, it is not great enough to interfere with estrous activity and normal cyclic rhythm. Cyclic rhythm may be altered by the physical contents of older degenerating embryos in the uterus, but when the embryos are younger there is no evidence of any change. Fernandez-Cano (1958) has presented evidence in the rat, showing that stress from increased temperature results in a pituitary-adrenal reaction. Increased body temperature caused embryo degeneration in intact rats, but not in adrenalectomized rats maintained on cortical implants. Velardo (1957) also found that embryo degeneration occurred in rats after administration of

adrenocorticotrophic hormone in intact rats but not in adrenalectomized rats. Critical data are not available for determining whether the detrimental effect is the result of one or a combination of the above possibilities.

#### SUMMARY AND CONCLUSIONS

The effect of high ambient temperature ( $32^{\circ}\text{C}$ ) on fertility and early embryo mortality was determined in ewes. Fertility rate in ewes exposed to high temperature on the twelfth day of the cycle and bred at the next estrus was significantly lower than for controls (40.7% vs. 94.2%). In ewes exposed to the elevated temperature at time of breeding, 69.2% of the ova were cleaved, and in ewes exposed 24 hr after breeding all ova were cleaved. Twenty-four hours after exposure to the elevated temperature rectal temperatures of the ewes increased on the average  $1.7^{\circ}\text{C}$ .

Exposing the ewes to heat resulted in an increase in morphologically abnormal ova. Only 3.8% of ova from control ewes examined 3 days after breeding were classified as abnormal. In the before-breeding group 55.6% were abnormal, and in the 1-day group all ova were cleaved, but 30.8% were abnormal.

In the control group 87.5% lambed, but none of the ewes exposed to the high temperature before or at time of breeding lambed. Of ewes exposed to high temperature at 1, 3, 5 or 8 days after breeding, 20%, 35%, 40% and 70%, respectively, lambed. Percent of ewes lambing was significantly lower in all groups exposed to the elevated ambient temperature except the 8-day group.

Embryo mortality, estimated as the percent of fertilized ova that failed to survive, was significantly higher for all treated groups except the 8-day group. Embryo loss was significantly higher in ewes exposed to high temperature before or within 24 hours after breeding than in ewes exposed after the embryo had migrated to the uterus (3, 5 and 8 days after breeding). The sheep zygote is most sensitive to harmful effects of thermal stress during the initial stages of cleavage before it enters the uterus and prior to the blastocyst stage.

When the embryo dies while it is still in the oviduct, the ewe usually returns to estrus within the normal interestrus period. The post-breeding estrous cycle is more likely to be of abnormal length if death is induced after the embryo enters the uterus.

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#### ABSTRACT

Percent of ova fertilized was significantly ( $P < 0.01$ ) lower in ewes exposed to  $32^{\circ}\text{C}$  temperature on the twelfth day of the cycle before breeding (40.7% vs. 94.2% for controls). The average rectal temperature of the ewes was increased  $1.7^{\circ}\text{C}$  within 24 hr after exposure to the elevated temperature. Morphologically abnormal ova were increased from 3.8% in control ewes to 55.6% in treated ewes. In ewes exposed to the  $32^{\circ}\text{C}$  one day after breeding all ova were cleaved, but 30.8% were morphologically abnormal. In control ewes 87.5% lambed, but none of the ewes exposed to  $32^{\circ}\text{C}$  before or at time of breeding lambed. In ewes exposed to  $32^{\circ}\text{C}$  at 1, 3, 5 or 8 days after breeding, 20%, 35%, 40% and 70%, respectively, lambed. Embryo mortality was significantly ( $P < 0.01$ ) higher for all treated ewes except those in the 8-day group. The sheep zygote is most sensitive to the harmful effects of high ambient temperature during the initial stages of cleavage before it enters the uterus.

#### ZUSAMMENFASSUNG

Die Zahl befruchteter Eier von Schafen, die vor dem Decken am 12. Tage des Zyklus einer Temperatur von  $32^{\circ}\text{C}$  exponiert wurden, war signifikant niedriger (40,7%) als bei Kontrolltieren (94,2%). 24 Stunden nach Übergang in die höhere Temperatur war die Rektaltemperatur  $1,7^{\circ}\text{C}$  höher als vorher. Der Anteil morphologisch abnormaler Eier bei den Kontrollen betrug 3,8%, bei den exponierten Tieren 55,6%. Bei Tieren, die einen Tag nach dem Decken  $32^{\circ}\text{C}$  exponiert wurden, zeigten alle Eier Teilung, doch davon waren 30,8% morphologisch abnormal. Von den exponierten Tieren lammt keines, von den Kontrolltieren 87,5%. Von Schafen, die 1, 3, 5 oder 8 Tage nach dem Decken  $32^{\circ}\text{C}$  exponiert wurden, trugen 20, 35, 40 und 70% aus. In allen exponierten Gruppen war die Mortalität signifikant höher ( $P < 0,01$ ) - ausser in der 8-Tage Gruppe - als bei Kontrollen. Die Zygote des Schafes ist äusserst empfindlich gegen hohe Temperatur während der Initialstadien der Teilung, ehe sie in den Uterus gelangt.

## RESUME

La proportion d'ovules fécondés a été trouvée significativement moindre ( $P < 0,01$ ) chez des brebis exposées à une température de  $32^{\circ}\text{C}$  lors du 12ème jour du cycle précédant l'insémination que chez des animaux-témoins (40,7% contre 94,2%). Pendant les 24 heures consécutives à l'exposition à la chaleur, la température rectale des brebis dépassait de  $1,7^{\circ}\text{C}$  sa valeur au cours de la période précédente. La proportion d'ovules morphologiquement anormaux était de 3,8% chez les animaux-témoins contre 55,6% chez les animaux exposés à la chaleur. Chez des animaux qui avaient été exposés à une température de  $32^{\circ}\text{C}$  le lendemain de l'insémination, tous les oeufs étaient segmentés, pourtant 30,8% d'entre eux présentaient des anomalies morphologiques. Des brebis ainsi exposées, aucune n'a conduit sa gestation à terme alors que 87,5% des animaux-témoins ont agnelé. Des brebis exposées à la chaleur 1, 3, 5 ou 8 jours après l'insémination, respectivement 20, 35, 40 et 70% ont mené leur gestation à terme. Dans tous les groupes exposés, la mortalité embryonnaire a été significativement plus élevée ( $P < 0,01$ ) que chez les témoins, à l'exception toutefois du groupe exposé 8 jours après l'insémination. Dans l'espèce ovine, le produit de la fécondation est très sensible à l'hyperthermie pendant les premiers stades de la segmentation qui précèdent son entrée dans l'utérus.