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FERTILITY IN THE RAM FOLLOWING EXPOSURE TO ELEVATED AMBIENT TEMPERATURE AND HUMIDITY

B. HOWARTH, JR*

National Institute of Child Health and Human Development, Bethesda, Maryland, U.S.A.

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Recent studies have demonstrated a loss of potential young as a result of heat stress on ejaculated spermatozoa before fertilization. Rabbit spermatozoa were capacitated for 6 hr in the uteri of does maintained at 32° C air temperature, recovered, and used to inseminate females maintained at 21° C; the spermatozoa retained their fertilizing capacity but contributed to subsequent embryo mortality before implantation (Howarth, Alliston and Ulberg, 1965). Ulberg & Burfening (1967) have shown a similar reduction in pre-implantation embryo survival for rabbit ova fertilized by spermatozoa cultured *in vitro* for 3 hr at 40° C, indicating a direct effect of heat on the spermatozoa.

This investigation was conducted to determine the effect of whole body exposure of mature rams to elevated ambient temperature in terms of both semen production and fertility during the initial 3-week period following treatment. Of interest was the determination of whether spermatozoa could be influenced by increased body temperature in such a way that they would initially retain their fertilizing capacity but contribute to subsequent embryonic mortality.

Eight experimental rams were kept for a period of 4 days in a temperature–humidity controlled room and exposed to an elevated ambient temperature of 32° C and relative humidity of 65%. Eight other rams served as controls and were maintained throughout the experiment under ambient temperature conditions. Before and following the treatment period, the experimental rams were maintained under the same conditions as the control rams. The average daily minimum and maximum outside temperatures during the experiment were 1·1° C and 12·8° C respectively. Fertility of control and experimental rams was evaluated in test ewes mated to individual rams. Each ram was mated to a pair of ewes weekly. One ewe of each pair was killed 30 to 40 hr after the end of oestrus to determine the rate of ovum fertilization. Fertilization was evaluated on the basis of at least one cleavage division. The other ewe of each pair was used to determine embryonic survival. Any of these ewes that returned to oestrus earlier than 34 days after mating were not killed; they were

* Present address: Department of Poultry Science, The University of Georgia, Athens, Georgia, U.S.A.

considered to have no surviving embryos. All ewes not returning to oestrus by 34 days were killed and embryonic survival was based on the presence of a heartbeat in the embryo. Due to a shortage of ewes and a lack of ovum fertilization during the 2nd and 3rd weeks in test ewes mated to the first five experimental rams, fertility was obtained on only six control and six experimental rams during the 2nd week and on five control and five experimental rams during the 3rd week following treatment.

Semen was collected from all rams on two occasions before treatment, once immediately following treatment and thereafter at weekly intervals for 3 weeks. Semen volume was measured to the nearest 0·1 ml. Percentage of motile cells was estimated at 10% class intervals by examining a drop of diluted semen at a magnification of ×200 with a phase contrast microscope. Concentration of sperm cells and percentage of morphologically abnormal spermatozoa was calculated by counting in a haemocytometer.

TABLE 1

AVERAGE RECTAL TEMPERATURES AND RESPIRATION RATES FOR CONTROL AND EXPERIMENTAL RAMS BEFORE, DURING AND AFTER THE TREATMENT PERIOD

Rams	*Before treatment*	*Days during treatment period*				*After treatment*
		1	2	3	4	
Rectal temperatures (°C)						
Control	39·3	—	—	—	39·4	39·4
Experimental*	39·3	40·2	39·9	39·8	39·8	39·2
Respiration rates/min						
Control	40	—	—	—	49	36
Experimental*	37	153	140	136	137	34

* Rectal temperatures and respiration rates were significantly ($P<0·01$) higher at all four readings during the treatment period than those before or after treatment.

Upon exposure to the experimental temperature (32° C) and humidity (65%), the rectal temperatures of experimental rams initially increased an average of 0·9° C. Analysis of variance and the use of multiple range tests showed that the mean rectal temperatures and respiration rates of experimental rams were significantly ($P<0·01$) higher on all 4 days of the treatment period than those recorded before or after treatment (Table 1).

Differences in fertilization and embryonic survival between control and experimental groups of ewes (Table 2) were tested for significance using tables of exact probabilities (one-tailed test) for 2×2 contingency tests (McGuire, Lehmann & Heath, 1967). In ewes mated the 1st week following treatment of the rams, differences between control and experimental ewes were not significant either for the fertilization rate or embryonic survival. Observations during the 2nd and 3rd weeks following the treatment period revealed a complete failure of fertilization in experimental ewes. However, two of the experimental ewes used for evaluating embryonic survival had viable embryos following mating during the 2nd week. This would indicate that ova successfully fertilized

TABLE 2

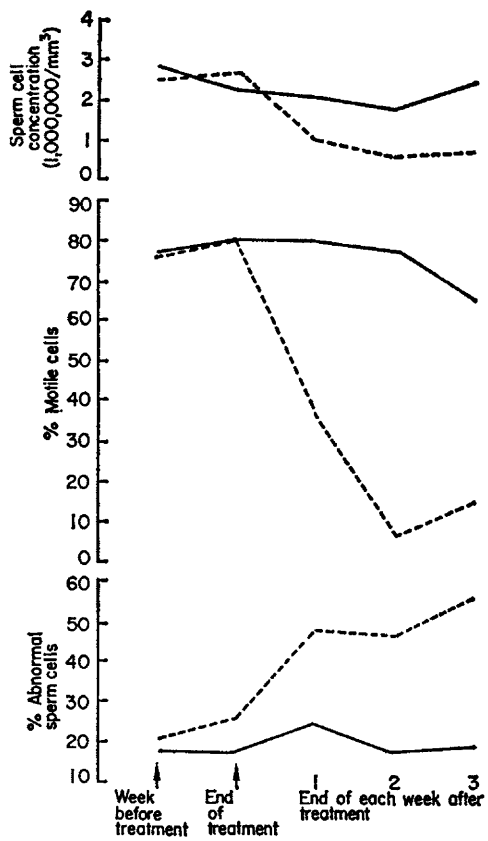
REPRODUCTIVE PERFORMANCE OF CONTROL AND EXPERIMENTAL RAMS

Treatment group	*Weeks following treatment*	*No. ewes with fertilized ova/ total no. ewes*	*No. ewes with viable embryos/ total no. ewes*
Control	1	7/8	7/8
	2	6/6	6/6
	3	5/5	3/5
Experimental	1	5/8	4/8
	2	0/5*†	2/6‡
	3	0/4*†	0/5

* Failed to recover ova in two test ewes; one during the 2nd week and one during the 3rd week following treatment of experimental rams.

† Fertilization rates for the experimental group were significantly ($P<0{\cdot}01$) lower than those for the control group during the same time period.

‡ Embryonic survival for the experimental group was significantly ($P<0{\cdot}05$) lower than the control group during the same time period.



TEXT-FIG. 1. Effect of exposing rams to elevated ambient temperature (32° C) and humidity (65%) upon various semen characteristics. ———, Control; – – – –, experimental.

during the 2nd week were capable of continued development. With the exceptions of one control and two experimental ewes, all ewes failing to conceive returned to oestrus 16 to 18 days after mating.

The drastic decline in fertility of experimental rams during the 2nd and 3rd weeks following treatment was associated with highly significant ($P<0{\cdot}01$) reductions in both sperm cell concentration and percentage of motile cells and a highly significant ($P<0{\cdot}01$) increase in the percentage of morphologically abnormal cells (Text-fig. 1). Volume of semen collected from experimental rams showed little or no change. Observations on sperm production were similar to those obtained by Dutt & Hamm (1957). The treatment had little or no observable effect on libido or sexual drive.

These results suggest that spermatozoa from experimental rams were not detrimentally affected by elevated temperature so as to cause an increase in subsequent embryonic mortality without affecting the fertilization rate. The relatively high rate of fertility observed in test ewes mated to experimental rams during the 1st week after treatment and the subsequent decline in fertility during the 2nd and 3rd weeks after treatment would indicate that spermatozoa of the epididymis were more resistant to the effects of heat than were spermatozoa of the testis. Waites & Ortavant (1967) have observed that B-type spermatogonia and pachytene spermatocytes at late stage 7 or early stage 8 were more temperature sensitive than other stages of spermatogenesis in rams whose testes were maintained at $40{\cdot}2\pm0{\cdot}2°$ C for 140 to 150 min. According to estimates of the duration of spermatogenic processes in the ram (Ortavant, 1959), approximately 36 days would be required for the expression of such damage to pachytene spermatocytes in terms of sperm quality at the time of emission. Gunn (1936) has demonstrated that only 5 to 6 days are required for the passage of spermatozoa through the epididymis of the frequently ejaculated ram. The deleterious effect of elevated temperature on sperm quality in the present study and the time of its expression (approximately 15 days after the beginning of the treatment period) would suggest that the observed low fertility was due to damage sustained during the late stages of spermatogenesis and would further attest to the apparent resistance of epididymal spermatozoa to the effects of heat. The resistance of epididymal spermatozoa to the effects of heat could be due to their lower metabolic rate or the absence of cellular division.

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