EFFECTS OF HIGH TEMPERATURE ON SPERM MORPHOLOGY AND SUBSEQUENT FERTILITY IN MERINO SHEEP

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Summary

An experiment was made to test the fertility of ram semen showing morphological abnormality due to exposure of the rams to high ambient temperature. Pyriform cells distinguishable by posterior constriction of the head, tailless sperm and an acrosomal cap abnormality were identified.

Three groups of 20 Merino ewes were each mated to two rams which had been heated for four days, or for two days, or unheated (control). Heat treatment was given for 8 h daily in a climate chamber at 40.5°C and 45% relative humidity. The percentage of abnormal spermatozoa increased and the fertility decreased with increasing duration of heat treatment. In the ewes mated to four-day heated, two-day heated and control rams respectively, 0, 15 and 60% of ewes lambed.

I. INTRODUCTION

Exposure of rams to high temperature leads to marked seminal degeneration (Phillips and McKenzie 1934; Dutt and Hamm 1957; and many others). Gunn, Sanders and Granger (1942) observed seminal degeneration when daily maxima exceeded 32.3°C. Moule and Waites (1963) found that as little as 12 h general body heating may be sufficient to induce seminal degeneration, while Waites and Setchell (1964) induced seminal degeneration by local heating of the testes to 40°C for 3 h. The emission of degenerate semen usually commences some two to three weeks after exposure to heat but may be delayed as long as four or five weeks (Dutt and Hamm 1957).

McKenzie and Phillips (1934) suggested 15 % of abnormal spermatozoa as the critical level for fertility in rams, while Cupps, Laben and Mead (1953) found a high correlation between the percentage of abnormal sperm and infertility. If relatively low percentages of abnormal spermatozoa are in fact associated with lowered fertility, it may well be that the presence of obviously abnormal cells also indicates a generalised disturbance of sperm production which renders ineffective a high percentage even of those spermatozoa which appear to be normal. Much probably depends on the type of abnormality and its origin, but there is little information available on this point.

Rathore and Yeates (1967) reported that heating of rams for two days (8 h daily) at 40.5°C resulted in the appearance, eight days post-heating, of pyriform sperm (i.e. cells showing posterior constriction of the head region). The pyriform heads were on average 0.711 μ shorter and 0.151 μ narrower ($P<0.01$) than the normal heads. The posterior third of the head showed a high reflectance, and the post-nuclear cap and axial filaments which were prominent features of the normal sperm were not distinguishable in the pyriform cells. This abnormality reached a

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peak 14-21 days post-heating and then declined abruptly, the appearance of the semen returning to normal after 28 days.

Not knowing the relevance of this abnormality to fertility, and in the hope of defining more precisely the marginal conditions associated with high temperature impairment of spermatogenesis, it was decided to conduct fertility trials with rams exposed to two levels of heating and whose semen contained known and differing proportions of pyriform cells, but which otherwise appeared normal and showed good motility.

II. MATERIALS AND METHODS

Six mature Merino rams, four experimental and two control animals, were used. Two of the experimental rams were exposed to hot chamber conditions for two days (8 h exposure daily) at 40.5°C with 45% relative humidity (R.H.); the other two were exposed to the same temperature for four days (also 8 h daily). The control rams were not heated. Semen was collected in an artificial vagina from all the rams on the day before treatment started, and every two days up to 20 days post-heating. A small quantity of the collected semen was withdrawn, and sperm concentration and motility were estimated. Duplicate smears were also made from each collection and stained with nigrosin-eosin as a test for "live" and "dead" sperm (Hancock 1951). The slides were dried on a warm plate (37°C) and mounted in D.P.X. The proportion of pyriform cells was estimated by counting 100 cells in each slide from fields representing the complete slide.

Seventy non-pregnant five-year old Merino ewes (of which 60 were finally mated) were treated with progesterone (20 mg/day intramuscularly every second day for 15 days) to synchronize oestrus. Vasectomised rams wearing marking harness (Radford, Watson and Wood 1960) were used to mark ewes as they came into oestrus, but in order to minimise any abnormal influences of the exogenous progesterone, fertile mating was delayed until the next cycle. Accordingly, as each ewe was marked at the second oestrus, she was hand mated to a ram from one of the three groups, viz:-Group 1: Two rams untreated (control): 20 ewes. Group 2: Two rams exposed to heat for two days (8 h daily): 20 ewes. Group 3: Two rams exposed to heat for four days (8 h daily): 20 ewes.

The whole operation was timed to ensure that mating coincided with the 10-16 day' post-heating period of the rams, and the ewes were allotted to the three groups at random, 10 ewes being mated to any one particular ram.

Following mating, the ewes were run with harnessed vasectomised rams and service marks were recorded daily. On day 12 post-mating, the ewes underwent laparotomy (Lamond and Urquhart 1961) to confirm ovulation and to assess the percentage of twin ovulations. On day 40 ± 3, those ewes which had not returned to service again underwent laparotomy to test for pregnancy.

III. RESULTS

Table 1 summarises data on the volume, density and morphological characteristics of semen from the control and treatment groups. The table shows that before heating the groups were fairly similar in ejaculate volume and density. After heating, however, volume declined in the heated rams, though the density was unchanged.

TABLE 1

Semen Characteristics (Group means)

			Control	2-day heated	4-day heated
Before heating	Ejaculate Volume (ml)		1.3	1.3	1.2
	Density (x 10^6/ml)		3250	2565	2480
8-16 days post-heating	Ejaculate Volume (ml)		1.3	1.2	0.9
	Density (x 10^6/ml)		3300	2610	2500
	Live cells	(%)	72	65	60
	Pyriform cells	(%)	0	25	35
	Tailless sperm	(%)	6	13	30
	Acrosomal abnormality	(%)	0	0	25

In the heated groups, the pyriform cells appeared eight days after the end of treatment. In the four-day heated group, the pyriform cells gradually increased, reaching a peak of 35 %10-1 6 days post-heating. About 30% of the pyriform cells took up the eosin stain, but the remainder appeared to be non-stained. There was an increase in the percentage of tailless sperm, reaching 30% on day 16.

A further characteristic feature noted was the partial detachment of the acrosomal cap from the sperm head. The incidence of this abnormality reached a peak of 25% 8-16 days post-heating.

In the two-day heated rams, pyriform cells reached a peak of 25% between 8 and 16 days. A further 12-15 % of the sperm were tailless but no acrosomal abnormality was found.

The proportion of ewes which had not returned to service by day 23, the number of ewes diagnosed as pregnant when laparotomized at 40 days post mating, and the lambing performance are shown in Table 2.

Analysis by chi-square showed that there was a high degree of homogeneity between the rams within each group. When each treatment group was compared with the control group, the differences in fertilization rate based on 23 day non-return were highly significant ($P<0.005$). Similarly, the numbers pregnant at 40 days differed significantly between all groups ($P<0.005$).

In this experiment, 10 out of the 60 ewes mated had two ***corpora*** *lutea,* while the other 50 had only one. With so few animals, a low percentage of double ovulations and many embryonic deaths, it was not possible to distinguish any difference in these results between single and twin ovulating ewes.

TABLE 2

Reproductive performance of ewes mated to treated rams

Ewe performance	Ram Treatment		
	Control	2-day heated	4-day heated
23 day non-return*	14/20	9/20	3/20
40 days pregnant*	12/20	3/20	0/20
Ewes lambed	12/20	3/20	0/20

*All groups significantly different ($P<0.005$).

IV. DISCUSSION

The results of this experiment show a close association between heat treatment, sperm morphology and fertility, in that the longer the period of heating the higher the incidence of pyriform and other abnormal cells and the lower the fertility. In four-day heated rams, only three of 20 ewes were still apparently pregnant after 23 days, and although this was associated with a high percentage of pyriform cells (35%), the percentages of non-pyriform sperm with an abnormal acrosomal cap and of tailless sperm were also high. Blom (1945) was able to relate the proportion of sperm with detached acrosomes in bull semen to fertility.

In the two-day heated rams, the fertility was still low (nine of 20 ewes apparently pregnant after 23 days), and it is perhaps significant that in this case no acrosomal abnormality was evident; nor was there a high proportion of tailless sperm. This result suggests that the pyriform condition is in fact a serious abnormality.

Further comparable work (Rathore, unpublished data) has shown that three-day heated, one-day heated and control rams respectively gave 20%, 60% and 70% of ewes apparently pregnant after 23 days.

Of the 20 ewes mated to four-day heated rams, three were apparently pregnant based on 23 day non-return to service. These three animals had not returned to heat by day 40 ± 3, at which stage pregnancy test by laparotomy confirmed earlier pregnancy but indicated current resorption which was confirmed by the ewes' subsequent return to service on days 47, 55 and 62 respectively, post-mating. Apparently the semen was capable of fertilizing the ova but not of maintaining pregnancy. This is of interest in view of the findings of Zorgniotti and Hotchkiss (1965) that repeated miscarriage in humans may be associated with a high proportion of abnormal forms in the semen.

In the two-day heated rams, nine of 20 ewes were apparently pregnant on day 23, but by day 37, six of the nine ewes had returned to service. On day 40 ± 3, the three remaining ewes were confirmed pregnant at laparotomy and subsequently lambed. In the six ewes which returned to service between day 23 and 37, embryonic death probably occurred sometime between day 15 and day 19 post-mating (Edey 1967). Again there is a suggestion, therefore, that sperm which were capable of fertilization, albeit at a low level, may not have been adequate in some respect for the maintenance of pregnancy.

The results of the present experiment demonstrate that a marginal degree of abnormality in sperm morphology, due to exposing rams to moderate heat, was closely associated with a depression in conception rate, and in the ewes which did conceive, there was probably a high incidence of embryonic death.

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