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SEMEN QUALITY IN BUCKS EXPOSED TO 34°C FOR 8 HOURS ON EITHER 1 OR 5 DAYS

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ABSTRACT

An experiment has been conducted on semen quality in male NZW rabbits exposed to 34°C for 8 hours on either 1 or 5 days.

Eight male NZW bucks were employed and divided into 2 groups. The first group was subjected to a single 8-hour exposure to 34°C; the second to 5 consecutive 8-hour exposures at the same temperature. During each hotroom exposure, respiration rate (RR), rectal temperature (RT) and scrotal temperature (ScT) were measured hourly. It was found that RT, RR and ScT increased with increasing time of exposure in both groups 1 and 2, particularly in the first 2 or 3 hours of exposure, and that this was followed by a significant increase in the percentage of dead sperm in ejaculates from bucks in both treatments ($P < 0.05$), and over weeks ($P < 0.001$).

Study of the incidence of pyriform cells revealed non-significant differential effects of temperature (1x8h vs 5x8h), but significant effects ($P < 0.001$) of weeks. In group 1, the % pyriforms increased to a peak of 3.8% in the first week after hotroom exposure, and in group 2, the 5x8h treatment, the pattern of change was very similar but the number of pyriforms was slightly higher (at 16%) than in 1x8h treatment.

It is concluded that an increase in the level of heat stress experienced by NZW bucks was followed by significant increases in the % dead and % pyriform sperm.

INTRODUCTION

In a previous experiment (KASA, 1991), bucks were exposed for 8 hours to either 32°C, 32°C + 1.9 MJ/m^2/h radiant heating, or 34°C. Significant differences in semen volume, density, motility and % dead sperm were observed after 34°C treatment, with the maximum influence observed 1 week after treatment. Semen characters returned to normal after 2-4 weeks. By way of contrast, in rams, BRADEN and MATTNER (1970) identified a marked depression in the number of spermatozoa in the ejaculate (to as low as 100 x 10^6/ml) between 34 and 47 days after exposure to 40.5°C. Buck rabbits and rams in those two experiments were exposed to quite different environmental temperatures, but were reported to experience similar increases in body temperature.

The latter finding suggests that the testes of buck rabbits may be more resistant to elevated temperatures than those of the ram, though differences in the heat treatments imposed may also be involved.

MATERIALS AND METHODS

Eight male NZW rabbits were randomly allocated to 2 groups of 4 in descending order of density of semen collected on 29/3/1989. The rabbits were kept in a control room at 20°C for two weeks before hotroom treatment. The bucks were then put in a hotroom at 34°C environmental temperature (ET) as follows:

1. Group I: a single 8-hour exposure from 09.00 to 17.00 h on April 10, 1989.
2. Group II: 5 consecutive 8-hour exposures to the same 34°C environment, from 09.00 to 17.00 h on each of the days from April 6 to April 10, 1989.

During each hotroom exposure, respiration rate (RR), rectal temperature (RT) and scrotal temperature (ScT) were measured hourly. The same measurements were also made hourly in the control room (20°C) at 18.00 h to 20.00 h on each day of hotroom exposure in order to monitor the animal's recovery from hyperthermal conditions.

The experiment ran for 8 weeks, with the animals fed and watered *ad-lib*. All bucks had been trained to serve the artificial vagina (MACIRONE and WALTON, 1938), and throughout the experiment semen was collected once weekly. The proportion of live and dead sperm was calculated from direct microscopic counting of 200 cells in nigrosin/eosin stained smears (BUTTLE et al., 1965). As an expansion of the semen evaluation, routine counts of the percentage of pyriform cells (RATHORE and YEATES, 1967) were made, based on 200 randomly selected spermatozoa in the eosin/nigrosin smear made from each ejaculate. Data on RT, RR and ScT were analysed using analysis of variance; the data on live and dead sperm, and % pyriform by analysis of covariance.

RESULTS AND DISCUSSION

Rectal Temperature

In group 1, RT increased very rapidly during the first 3 hours of exposure to reach a plateau level of 41.0 to 41.3°C which was maintained until the 8th hour. When returned to 20°C at the end of the 8th hour, RT declined to pre-treatment levels within 2 hours (Fig.1).

In group 2, mean RT increased most rapidly during the first 2 hours of exposure (Table 1 and Fig.1); from 39.2 ± 0.1 to 40.1 0.2°C. Thereafter, it continued to rise slowly and significantly ($P < 0.05$), but by only another 0.6°C in the remaining 6 hours in the hotroom. Differences in RT between rabbits were significant (range from 39.5° to 40.5°C), as were those between days (both $P < 0.001$; Table 1). The decline in mean RT from day 1 to day 4 (from 40.5° to 39.6°; SEM = 0.01°C) was progressive (Table 1), and suggested that the bucks were gradually acclimating to the repeated daily hotroom exposures. On day 5, however, a slight (0.3°C) but significant rise in mean RT occurred, indicating, perhaps, that short-term acclimation had stabilized at about that time. Longer term observations would

have been necessary to clarify that point.

Table 1. Mean rectal temperature (°C) of male NZW rabbits exposed to 34°C for 8h on 5 consecutive days

	Rectal Temperature (°C)	SEM	Level of Significance
Rabbit (1-4)	39.9a 39.5b 40.1c 40.5d	0.01	***
Day (1-5)	40.5a 40.1b 39.9c 39.6d 39.9c	0.01	***
Time of Exposure (0-10 Hours)	39.2a 39.8b 40.1c 40.3d 40.4e 40.5f 40.6g 40.6g 40.7h 39.8b 39.2a 39.1i	0.01	***

Values within the same line with dissimilar superscripts differ significantly ($P < 0.05$)

Respiration Rate

Data on RR are presented in Table 2 and in Fig. 1. In group 1, respiration rate increased rapidly (7 fold) during the first 3 hours of exposure and then maintained a plateau level of about 400 breaths/minute until hour 8. When the rabbits were then returned to the control room at 20°C, RR rapidly decreased to the pre-treatment levels within one hour (Fig. 1). Overall, in group 2, RR also increased most rapidly (5 fold) in the first hour of exposure and then maintained a plateau level of about 300-400/minute until hour 8. There were significant variations in RR between hours 1 and 8, but these were relatively small in magnitude (less than 35 breaths/minute in any one hour-to-hour comparison), and of doubtful biological significance in view of the acknowledged difficulty of accurately counting high RR values by eye over a short time interval. When the animals were returned to the control room, RR decreased as rapidly as it had risen in the first hour of hotroom exposure, and by hour 9, RR was back to pre-treatment levels on all days except day 1 (Fig. 1). A similar pattern was followed on each of the 5 experimental days (Table 2 and Fig. 1). Examination of Fig. 1 and Table 2 indicates a progressive reduction in the RR response from days 1 to 5. Even with the difficulties of accurately estimating RR (and these would not have been great at 200-300 breaths/minute),

Table 2. Mean respiration rate (breaths/minute) of male NZW rabbits exposed to 34°C for 8h on 5 consecutive days

	Respiration Rate (breaths/minute)	SEM	Level of significance
Rabbit (1-4)	256.3a 252.0b 247.6a 268.5c	0.5	***
Day (1-5)	302.5a 278.9b 257.3c 226.8d 227.5d	0.5	***
Time of Exposure (0-10 Hours)	57.8a 286.5b 320.5c 338.8d 366.3e 367.3e 366.8e 378.8f 377.3f 115.0g 72.8h 55.5a	0.5	***

Values within the same line with dissimilar superscripts differ significantly ($P < 0.05$)

the overall magnitude of this day effect (a decline from 300/minute on day 1 to only about 226/minute on days 4 and 5) is strongly suggestion of acclimation. As with RT, the downward trend in RR was halted between days 4 and 5.

Scrotal Temperature

In group 1 (1x8h treatment), ScT increased very rapidly during the first hour of exposure by an average of 3.8°C (Fig. 1) and then reached and maintained a plateau level of 37.5 ± 0.2

Table 3. Mean scrotal temperature (°C) of male NZW rabbits exposed to 34°C for 8h on each of 5 consecutive days

	Scrotal Temperature (°C)	SEM	Level of Significance
Rabbit (1-4)	36.1a 35.8b 35.7c 36.4d	0.03	***
Day (1-5)	36.6a 35.9b 35.8bc 35.7c 35.9b	0.02	***
Time of Exposure (0-10 Hours)	32.4a 36.8b 37.4c 37.7d 37.7d 37.8de 37.9ef 38.0f 38.0f 33.5g 32.5a 32.1h	0.02	***

Values within the same line with dissimilar superscripts differ significantly ($P < 0.05$)

to 38.1 ± 0.2°C between hour 2 and 8. When the rabbits were returned to the control room, ScT declined to pre-treatment levels within 1 hour. In group 2, ScT differed significantly between days ($P < 0.001$; Table 3). The biggest response was on day 1 (mean 36.6°C) and then followed a progressive reduction to a value of 35.7°C or day 4, in an apparent acclimation pattern. Despite any such acclimation, the mean value in the hotroom (ie. excluding the pre-exposure figures taken at 20°C on day was 37.8 ± 0.1°C, which clearly indicates a severe and continuing level of thermal stress. Scrotal temperature rose rapidly during the first hour in group 2 (by 4.4°C; Table 3) and then maintained a virtually constant level of 37.4 - 38.0°C for the remaining 7 hours. The statistically significant variations of less than 0.3°C recorded on an hour-by-hour basis between hours 3 and 8 are likely to have been of little biological significance. Within 1 hour of being returned to the 20°C control room, ScT had returned to pre-treatment levels. Within the limits of precision of hourly measurements, it can thus be concluded (supported by the RT and RR data) that the testes of the bucks in the experiment were subjected to thermal stress (for example, ScT values in the hotroom of from 36.1 to 39.0°C) for 8 hours on each treatment day.

Percentage Dead Sperm

For the percentage of dead sperm in the ejaculate, analysis of covariance revealed significant influences of both treatment ($P < 0.05$) and weeks ($P < 0.001$). Figure 1 illustrates these variations, with values in weeks 1 and 2 being elevated ($P < 0.001$) and those during weeks 3 and 4 progressively declining (both $P < 0.05$). In week 5, the percentage of dead sperm did not differ significantly from pre-treatment values, although there is a trend evident in weeks 4, 5 and 6 of Fig. 1 for group 2 values to remain slightly elevated.

Between treatments, Fig. 1 indicates a substantially greater response in group 2 (5x8h hotroom exposure) than in group 1 (1x8h). This treatment effect was significant in weeks 1, 2 and 3 (each $P < 0.05$).

Table 4. Mean ± S.E. percentage of dead sperm in the semen of male NZW rabbits exposed to 34°C ET for different times (1x8 or 5x8h) at week 0

Week	1x8h	5x8h
0	0.0 ± 0.0	0.0 ± 0.0
1	14.5 ± 2.0	31.0 ± 7.0
2	10.3 ± 2.1	25.5 ± 4.1
3	6.6 ± 1.1	11.0 ± 0.9
4	1.8 ± 0.6	5.8 ± 1.0
5	0.6 ± 0.2	2.1 ± 1.0
6	0.5 ± 0.3	1.5 ± 0.8
7	0.4 ± 0.2	0.8 ± 0.3
8	0.3 ± 0.2	0.4 ± 0.1
Mean	3.8 ± 1.7	8.7 ± 3.7

It may be concluded from this study that the % dead sperm in rabbit semen increased at elevated ET, and with increasing severity of heat stress (1x8h at 34°C vs 5x8h). Since little work has been conducted on climatic effects on semen quality in rabbits, the only comparisons that can be made are with domestic animals such as sheep. RATHORE (1968) found that the incidence of dead spermatozoa rose to 35 and 40% after exposure of rams to 45°C ET for 2 and 4 days respectively. By way of comparison, dead spermatozoa rose to 17 and 25.5% respectively after 1 or 5 days of exposure of rabbits to 34°C in the current study. Obviously, the greater effect in sheep than in rabbits could have been due to the higher ET to which the former were exposed. Equally, however, it is possible that the testes of these two species respond differently to hyperthermia. Detailed comparative studies will be required to clarify this point, preferably designed in such a way that comparison can be made at similar testicular temperatures.

Percentage Pyriform Cells

The pyriform cell data presented in Table 5 and Fig.1 revealed non-significant effects of temperature (1x8h vs 5x8h), but significant effects ($P < 0.001$) of weeks. In group 1, the % pyriforms increased to a peak of 3.8% in the first week after hotroom exposure, and then declined to pretreatment levels by week 4. In group 2, the 5x8h treatment, the pattern of change was very similar (Fig. 1) but the number of pyriforms was slightly higher than in 1x8h treatment. In week 1, for example, the % pyriforms was 16% higher than in the 1x8h treatment group and overall, the mean pyriform cell count was 1.6 times higher in group 2 compared to the group 1. This finding is in general agreement with RATHORE's (1970) work in which NZW bucks exposed to 36.1°C ET and 45% RH for 7 hours on either 1 or 2 days were found to have 10% and 16% respectively of pyriform cells in their semen 16-23 days after treatment.

Table 5. Mean ± S.E of percentage of pyriform cells in the semen of male NZW rabbits exposed to 34°C ET, for different times (1x8 or 5x8h) at week 0

Week	1x8h	5x8h
0	0.0 ± 0.0	0.0 ± 0.0
1	3.8 ± 0.5	4.4 ± 0.5
2	3.3 ± 0.4	4.3 ± 0.3
3	1.9 ± 0.3	2.9 ± 0.7
4	0.1 ± 0.1	0.5 ± 0.4
5	0.3 ± 0.1	0.9 ± 0.3
6	0.1 ± 0.1	1.0 ± 0.7
7	0.0 ± 0.0	0.3 ± 0.1
8	0.3 ± 0.1	0.4 ± 0.1
Mean	1.1 ± 0.5	1.6 ± 0.6

In Merino sheep, on the other hand, RATHORE (1968) found 35% of pyriforms in the semen of rams heated (at 40.5°C) for 4-days and

25% when heating was for 2 days. Additionally, RATHORE's work showed corresponding differences in tailless sperm (30 and 13%) and acrosomal abnormalities (25 and 0%).

From the current results it can be concluded that the % pyriform cells in the ejaculate of heat stressed rabbits increases with time of hotroom exposure. RATHORE (1970) reported a similar trend, although the greater incidence of pyriforms in his work may have been due to the higher ET (36.1°C) used. Previous experiments on the effects of various ET (23.8 to 43.2°C) on physiological responses of NZW rabbits indicated that the current rabbits would have been unable to tolerate 36.1°C for 8 hours (KASA and THWAITES, 1990). The temperature used in the current work, 34°C, was estimated to be as high as the particular rabbits could tolerate for 8 hours, but even so the maximum incidence of pyriform cells was only 25.5%. These conflicting results would seem to suggest that RATHORE's rabbits had a higher level of general heat tolerance, but that their testes were more sensitive to heat. It is not possible to be more specific, however, since RATHORE collected only one ejaculate post-treatment, at 16-23 days. By that time the pyriform count in the current experiment had returned to the pretreatment level. If RATHORE's levels followed a similar post-treatment pattern to that observed here (i.e. quick increase followed by gradual decline), then the peak incidence of pyriforms in his bucks would presumably have been much higher than the 1-week figure in the current work.

Overall, it can be concluded that an increase in the level of heat stress was followed by significant increases in the % dead and % pyriform sperm. Semen density and volume were not significantly affected. These results confirm those of an earlier experiment, which indicated that 34°C ET was detrimental to semen quality KASA (1991), and clearly suggests that NZW bucks should be maintained at an ET of less than 34°C if seminal degeneration and adverse physiological affects such as high RT and RR are to be avoided. One of the difficulties encountered in this experiment was the lack of sufficient numbers of bucks. Between-animal variations in semen characters are large, so that future studies should concentrate on larger numbers of males. The current results would suggest that as many as 10 bucks/treatment is desirable. The current results also suggest that the rabbit's testes could be more resistant to high ET than those of the ram, even though it is possible that the rabbit's lower overall heat tolerance limits the amount of stress experienced by the testes. Detailed comparative studies are required to establish and elucidate these differences.

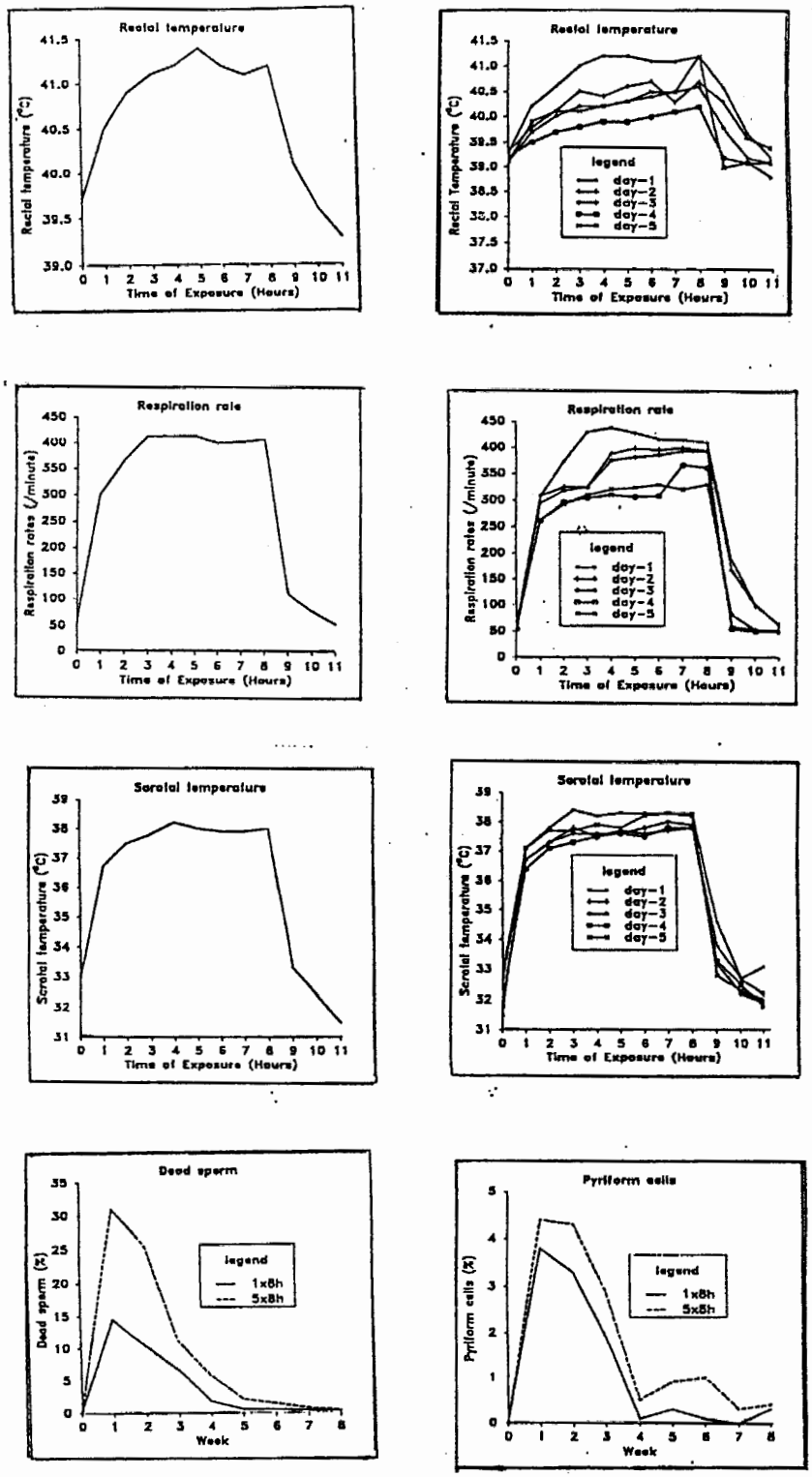

Fig. 1: Changes in rectal temperature, respiration rate, scrotal temperature, % dead sperm and % pyriform cells of male NZW rabbits exposed for 1x8h and 5x8h to 34°C ET and during subsequent 3-hour recovery periods at 20°C.

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