J U R N A L M E T A M O R F O S A
Journal of Biological Sciences
ISSN: 2302-5697
http://ojs.unud.ac.id/index.php/metamorfosa

Effect of Heat Exposure on Percentage of Dead Sperm and Pyriform Cell in Male New Zealand White (NZW) Rabbit

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ABSTRACT

Experiment has been done on the effect of heat exposure on the percentage of dead sperm and pyriform cell in male NZW rabbit. The bucks were exposed to 34°C for 8 hours on either 1 or 5 days. It was found that scrotal temperature (ScT) increased very rapidly during the first hour of exposure by 4°C in group 1 (1x8h). In group 2 (5x8h of exposure) ScT differed significantly between days ($P < 0.05$). The bigest response was on day 1 (36.6°C) and followed a progressive reduction, day by day, in an apparent acclimation pattern. At different times of exposure, ScT was observed to rise rapidly during the first hour (by 14 percent or 4.4°C). In the 1x8h treatment dead sperm achieved a maximum level of 14.5 ± 4.7 percent in the first week after hotroom treatment. In week 2, the percentage dead sperm started to decline. When rabbits were exposed to 5x8h, this group was more severely affected than the 1x8h. In week 1, the percentage dead sperm in the 5x8h group was 2.1 fold greater than in the 1x8h group (31.0 vs 14.5 percent). Recovery in the 5x8h group took longer than in the 1x8h group, and the percentage dead sperm finally returned to normal levels only after 7 weeks, compared to 5 weeks after 1x8h treatment.Between weeks, the incidence of pyriforms did differ significantly ($P < 0.05$). In the 1x8h treatment, the percentage pyriforms increased in the first week after hotroom exposure by 3.8 percent. In week 2, the percentage pyriform started to decline and continued to do so gradually until week 6. In the 5x8h treatment, the number of pyriforms was higher than it was in 1x8h treatment. In week 1, for example, the percentage pyriform was 16 percent higher than in 1x8h treatment group. Hence, the mean pyriform cell count was 1.6 times higher in the 5x8h compared to the 1x8h group.

Key Words : High temperature, dead sperm, pyriform, NZW rabbit.

INTRODUCTION

Studies on the effect of elevated temperature on semen quality and quantity have been conducted in rams, but very rare in rabbit buck. In ram most authors indicating that high temperatures adversely effect semen volume, motility, density and the proportion of live sperm (Dutt and Simpson, 1957; Moule and Waites, 1962; and Braden and Mattner, 1970). Subsequently, Braden and Mattner (1970) found that when ram's testes were heated 40.5°C for either 1.5 or 2 hours, or to 39.5°C for 4 hours, there was a marked depression in the number of spermatozoa per ejaculate between days 34 and 47. In rabbit buck, when treated with infrared heating, Kasa and Thwaites (2001) reported significantly different on sperm mortality in comparison to control. Therefore, this experiment was designed on the effect of heat exposure of 34°C for 8 hours on either 1 or 5 days in bucks of NZW rabbit.

MATERIALS AND METHODS

Eight mature NZW male rabbits (3049 ± 247g live weight) were randomly allocated to 2 groups of 4 in descending order of density of

semen collected. 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 temperature as follows:

1. Group I: a single 8-hour exposure from 09.00 to 17.00 h.
2. Group II: 5 consecutive 8-hour exposures, from 09.00 to 17.00 h on each of the days.

These animals were individually housed in new wire cages (46 cm x 30 cm x 28 cm) at 20°C in a temperature- and light-controlled room in another section of the animal-house complex. All animals in the experimental colony were identified by ear tattoo, and detailed records were kept. The bucks had been trained to serve the artificial vagina (Macirone and Walton, 1938). During each hotroom exposure, scrotal temperatures were measured using a non-invasive infra-red thermometer held at a distance of 20 cm from the scrotal using the built-in focussing system. The same measurements were also made hourly in control room of 20°C.

Semen evaluation:semen was collected and evaluated weekly. The proportion of live sperm was calculated from direct microscopic counting of 200 cells in negrosin/eosin stained smears (Buttle *et al*., 1965). From the negrosin/eosin smears the proportion of pyriform cells (referred to as "per cent pyriform") in a total count of 200 cells was obtained according to the technique described by Salamon (1976).

Feed and feeding:as a routine, whenever animals were maintained under non-experimental conditions and unless a specific nutritional treatment was applied, all animals were fed a standard ration ad libitum. Feeding took place in wall-mounted galvanized metal feeders with an anti-spill lip. Fresh rabbit pellets (0.05 to 1.75 cm long and 0.50 cm in diameter from Fielders Feedmill) were added to each feeder at 09.00 and 16.00 h daily and accumulations of dusty, broken pellets were removed whenever necessary. Feeders were removed and cleaned at 7-day intervals. Rejected pellets were discarded and fresh pelletsadded to start off the next weekly period.

Watering: each cage was fitted with an individual bottle drinker with a metal ball nipple (MADDOCK) 8.5 cm long and 3.5 mm in diameter; orientation and location were standardized between cages. The bottles were filled with fresh water daily and refilled if necessary. Bottles and nipples were cleaned throughly with detergent every 7 days. Any rabbit which exhibited an unusually low water intake, had its bottle and nipple changed immediately.

RESULTS

Scrotal Temperature (ScT): DataonScT are presented in Table 1. In group 1 (1x8h treatment), ScT increased very rapidly during the first hour of exposure by 4°C and then maintained a plateau level untill hour 8. When the rabbits were returned to the control room at 20°C, ScT declined to pre-treatment levels by hour 11. In group 2 (5x8h of exposure) ScT differed significantly between between days ($P < 0.05$).

Table 1. 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	32.4a	36.8b	37.4c	37.7d	37.7d	37.8de		
(0-10 hours)	37.9ef	38.0f	38.0f	33.5g	32.5a	32.1h	0.02	***

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

Table 2. Mean dead sperm ± S.E. (percent) of male NZW rabbits exposed to 34°C environmental temperature, at different times (1x8h or 5x8h) at week 0.

Week	1x8h	5x8h	mean
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1	14.5 ± 2.3	31.0 ± 7.8	22.8 ± 5.5
2	10.3 ± 2.5	25.5 ± 4.7	17.0 ± 3.6
3	6.6 ± 1.3	11.0 ± 1.0	8.8 ± 1.2
4	1.8 ± 0.7	5.8 ± 1.1	3.8 ± 0.9
5	0.6 ± 0.2	2.1 ± 1.1	1.4 ± 0.7
6	0.5 ± 0.4	1.5 ± 0.8	1.0 ± 0.6
7	0.4 ± 0.2	0.8 ± 0.3	0.6 ± 0.3
8	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Mean	3.0 ± 1.1	8.7 ± 2.1	6.0 ± 1.6

Table 3. Mean percentage of pyriform cells ± S.E. in the semen of male NZW rabbits exposed to 34°C environmental temperature, for different times (1x8h or 5x8h) at week 0.

Week	1x8h	5x8h	Mean
0	0.0 ± 0.0	0.0±0.0	0.0±0.0
1	3.8 ± 0.5	4.4±0.6	4.1±0.5
2	3.3±0.4	4.3±0.3	3.8±0.4
3	**1.4±0.4**	**3.9±0.7**	**2.4±0.6**
4	**0.1±0.1**	**0.5±0.5**	**0.3±0.3**
5	**0.3±0.1**	**0.9±0.4**	**0.6±0.3**
6	0.1±0.1	1.0±0.8	0.5±0.5
7	0.0±0.0	0.3±0.1	0.1±0.1
8	0.3±0.1	0.4±0.1	0.3±0.1
Mean	1.1±0.8	1.6±0.4	1.3±0.3

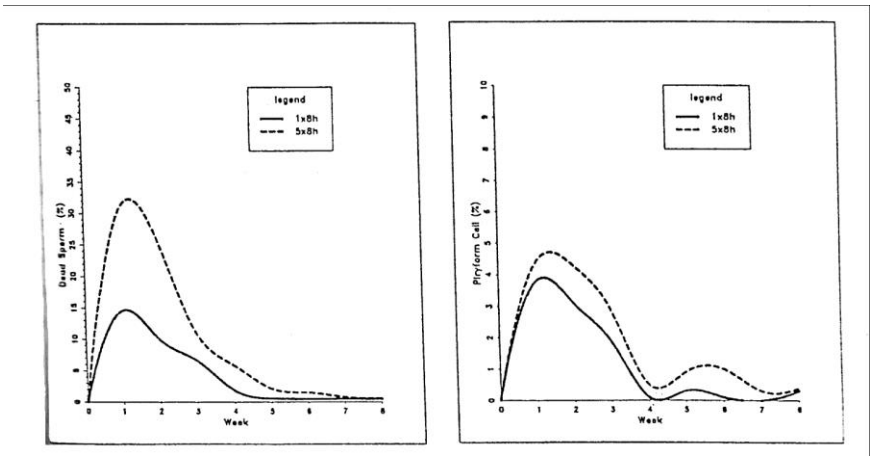

Fig. 1. Changes in percentage of dead sperm and pyriform cells of male NZW rabbits exposed to 34°C environmental temperature for different time (1x8h and 5x8h).

The bigest response was on day 1 (36.6°C) and followed a progressive reduction, day by day, in an apparent acclimation pattern. Mean values in the hotroom declined progressively to 35.7°C on day 5, indicating that while acclimation apparently occured the experimental condition represented a severe thermal stress on all days of treatment.

At different times of exposure, ScT was observed to rise rapidly during the first hour (by 14% or 4.4°C; Table 1) and then to maintain a virtually constant level of 37.4-38.0°C for the remaining 7 hours. The decrease in ScT when animal were returned to the 20°C control room was more rapid than the rise previously observed in the first hour of hotroom exposure (decline of 4.5°C compared to a rise of 4.4°C); after 3 hours in the control room ScT had returned to normal.

Percentage Dead Sperm:Data on percentage occurance of dead sperm in the ejaculates are presented in Table 2, Figs. 1 and 2. Between temperatures, the differences were significant ($P < 0.05$). In the 1x8h treatment dead sperm achieved a maximum level of 14.5 ± 2.3 percent in the first week after hotroom treatment. This represented an increase of 14.5 ± 2.3 percent. In week 2, the percentage dead sperm started to decline, which it continued to do progressively until the pre-treatment level was regained in the 5th week. The pattern was similar when rabbits were exposed to 5x8h, although this group was more severely affected than the 1x8h. In week 1, for example, the percentage dead sperm in the 5x8h group was 2.1 fold greater than in the 1x8h group (31.0 ± 7.8 vs 14.5 ± 2.3 percent). Recovery began in the 5x8h group in week 4, but the percentage dead sperm was then still higher (2.5 fold) than in the 1x8h group ($P < 0.05$). Recovery in the 5x8h group took longer than in the 1x8h group, and the percentage dead sperm finally returned to normal levels only after 7 weeks, compared to 5 weeks after 1x8h treatment (Table 2).

Percentage Pyriform Cells:Pyriform cell data are presented in Table3, Figs. 1 and 3. Between temperatures (1x8h vs 5x8h), the occurrence of pyriform cells did not differ significantly. Between weeks, on the other hand, the incidence of pyriforms did differ significantly ($P < 0.05$). In the 1x8h treatment, the percentage pyriforms increased in the first week after hotroom exposure by 3.8± 0.5 percent. In week 2, the percentage pyriform started to decline and continued to do so gradually until week 6. The normal level was regained by week 7 and 8. In the 5x8h treatment, the pattern of change was very similar but the number of pyriforms was higher than it was in 1x8h treatment. In week 1, for example, the percentage pyriform was 16 percent higher than in 1x8h treatment group. The decrease in level of pyriforms started in week 3 and by weeks 7 and 8 values were back to normal. Hence, the mean pyriform cell count was 1.6 times higher in the 5x8h compared to the 1x8h group.

DISCUSSION

In rabbits, the percentage dead sperm increased with elevated environmental temperature, and with increasing severity of heat stress (1x8h at 34°C vs 5x8h). Relatively little work has been conducted on semen quality in rabbits as influenced by climate. The only comparison that can be made are with amongst other domestic animals such as sheep. In sheep Rathore (1968) found that dead spermatozoa were 35 and 40 percent after exposure to 45°C environmental temperature for 2 and 4 days respectively. By way of comparison, in the current experiment on rabblts dead spermatozoa were 17 to 25.5 percent after 1 or 5 days of exposure to 34°C. The rabbits were exposed under 34°C environmental temperature and they could tolerate this temperature for 8 hours. Therefore, the greater effect on sheep than rabbits could be due to the higher environmental temperature which was employed on sheep. It could be also because sheep have more insulation (thicker fleece) so that heat loss through evaporative cooling (sweating) is not so effective.

The result in pyriform cell is in general agreement with Rathore's (1970a) work in which NZW bucks were exposed to 36.1°C environmental temperature and 45 percent relative humidity for 7 hours on either 1 or 2 days and 16-23 days post heating the number of pyriform cells was 10 percent in the group heated on 1 day only, whereas in the 2-day group it was 16 percent. In Merino sheep, for comparison, Rathore (1968) found that the percentage pyriform was 35 percent in 4-day heated and 25 percent in 2-day heated when exposure was conducted under 40.5°C and 45

relative humidity. Moreover, Rathore's work showed that the percentage tailless sperm was 30 and 13; percentage acrosomal abnormalities 25 and 0 respectively in 4-day and 2-day heated group.

From the current results it can be suggested that the percentage pyriform cells in the ejaculate of heat stressed rabbits increased with time of exposure in the hotroom. Rathore reported a greater incidence of pyriform cells than that observed in the current work, possibly due to the higher environmental temperature (36.1°C) employed in his work. The rabbits used in this experiment, which were of a different strain to those used byRathore (1970b), would have been unable to tolerate 36.1°C for 8 hours. The hotroom exposure employed 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 percent. 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 0.3 percent, the normal 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 pyriform in this bucks would presumably have been much higher than the 7-day figure in the current work.

CONCLUSIONS

Overall, it can be concluded that an increase in the time of hotroom exposure was followed by a significant degree of seminal degeneration; a rise in percentage dead sperm and percentage pyriform. The current results also suggest that the rabbit's testes could be more resistant to high environmental temperature than those of the ram, eventhough it is possible that the rabbit's lower overall heat tolerance limits the amount of stress experienced by the testes.

ACKNOWLEDGEMENTS

The author wishes to express sincere appreciation to the International Development Programme (IDP) of Australia for the fund which made this study possible as well as the Department of Animal Science, University of New England, Armidale, NSW, Australia for the facilities.

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