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Effect of dietary restriction on sperm characteristic and oxidative status on testicular tissue in young rats exposed to long-term heat stress

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Summary

This study was conducted to evaluate the effects of dietary restriction on oxidative status and sperm parameters in rats exposed to long-term heat stress. Forty healthy Sprague–Dawley rats, aged 2.5 month, were divided into four groups of 10 with respect to feeding and temperature regimen (room temperature (22 °C)-*ad libitum*, room temperature–dietary restriction (40%), high temperature (38 °C)-*ad libitum*, high temperature–dietary restriction). At the end of the 9th week, some oxidants (lipid hydroperoxide, total oxidant status, oxidative stress index) and antioxidants (total antioxidant status, sulfhydryl groups, ceruloplasmin, paraoxonase and arylesterase activities) were measured in the testis tissue. The concentration, motility, volume, abnormal sperm count, acrosome and membrane integrity of epididymal spermatozoon and intratesticular testosterone levels were evaluated. High temperature did not change oxidative and antioxidative parameters except for sulfhydryl groups and ceruloplasmin, yet it impaired all sperm values. Neither sperm values nor oxidative status apart from sulfhydryl groups, ceruloplasmin and arylesterase was affected by dietary restriction in the testis tissue. These results suggest that long-term heat stress does not have a significant effect on testicular oxidative status, while the spermatozoa are sensitive to heat stress in young rats. Dietary restriction failed to improve the sperm quality and oxidative status except some individual antioxidant parameters; conversely, it decreased intratesticular testosterone level in the young rats exposed to long-term heat stress.

Introduction

Male reproductive health has declined with marked increase in the population of subfertile males in the past decade. One of the possible suggested reasons of male infertility is prolonged exposure of the testis to high temperatures (Dada *et al.*, 2001). Scrotal temperature in mammals is a few degrees lower than that of the abdomen, and this cooler temperature is essential in order for the testis to maintain an optimal environment for spermatogenesis. Impaired testicular thermoregulation causes testicular dysfunction and disruption of spermatogenesis, resulting in temporary or permanent infertility (Ikeda *et al.*, 1999; Thundathil *et al.*, 2012). It was suggested that this degenerative process of testicular germ cells by heat stress involves impaired oxidative metabolism, transcription, RNA processing, translation, membrane structure, and function (Iwagami, 1996). When the testis is exposed to stress, it stimulates a variety of mechanisms such as DNA repair, heat shock response, oxidative stress response, apoptosis and cell death (Kanter *et al.*, 2011). Like all cells living under aerobic conditions, testis tissue and spermatozoa constantly produce reactive oxygen species (ROS) as an integral part of normal metabolism (Sharma & Agarwal, 1998). Low and controlled concentrations of these ROS are important in the regulation of sperm physiological processes such as capacitation, acrosome reaction, stabilisation of mitochondrial capsule and signalling processes to ensure fertilisation (Bansal & Bilaspuri, 2011). However, mammalian testicular

membranes are rich in polyunsaturated fatty acids and highly susceptible to oxidative stress. Accordingly, a higher generation of ROS can lead to male infertility by facilitating several pathogenic mechanisms such as varicocele, cryptorchidism, infections, obstructive lesions, tumours, defective sperm function and testicular damage (Agarwal *et al.*, 2008; Bansal & Bilaspuri, 2011). There have been many different attempts to alleviate the negative effects of high environmental temperature on male fertility such as modifying the environment, building, breeding and nutritional practices. These attempts have been focused mostly on dietary manipulation. One of the dietary attempts, diet restriction (DR), has been shown to extend the lifespan of various laboratory animals. A possible benefit of DR is a reduction in oxidative stress. Some researchers have measured a lower oxidative stress in various organs in diet-restricted animals compared with animals fed *ad libitum* (AL) (Dubnov *et al.*, 2000). In particular, the most of beneficial effects of DR on oxidative status were obtained in aged animals (Wu *et al.*, 2003). On the other hand, Sitzmann *et al.* (2010) reported that DR has no detrimental effects on testicular function and sperm values in *rhesus macaques*. In addition, DR may even benefit reproductive fitness through a reduction in body mass and adiposity, which has been shown to have significant effects on the reproductive axis. For example, it is known that obese men exhibit lower sperm concentrations compared with men having a normal body mass index (Kort *et al.*, 2006). This study suggested that DR would enhance heat tolerance by reducing the cellular oxidative stress and subsequent accrual of injury in testis. To our knowledge, there are no data that have investigated the potential protective role of DR on sperm parameters and oxidative status in testis of rats exposed to long-term high-temperature stress. Therefore, this study was conducted to determine effect of long-term heat stress on testicular oxidative stress and sperm parameters and whether DR would improve the ability of young rats to tolerate the effects of heat stress on testis tissue.

Materials and methods

Animals, diets and experimental design

All animal experimental procedures were approved by Harran University Animal Experimentation Local Ethics Committee (38/7, 13.01.2012). A total of 40 healthy Sprague–Dawley rats, aged 2.5 month, weighing 315 ± 15 g, were acclimated to the environment for 2 weeks. The rats were weight-matched according to their initial body weights and divided into four treatment groups of 10 rats each. The rats were placed in two controlled environmental temperature chambers (room temperature, RT (22 °C); and high temperature, HT (38 °C)). Group 1 and Group 2 were reared at a room temperature of 22 °C. Group 1 (RT-AL) was fed *ad libitum*, while Group 2 (RT-DR) had 60% of the diet consumed by Group 1. Group 3 and Group 4 were reared at 38 °C. Group 3 (HT-AL) was fed *ad libitum*, while Group 4 (HT-DR) was fed with 60% of the diet consumed by Group 3. The level of feeding was determined for each individual and was characterised by uneaten chows remaining in the animal's cage at the end of each day. Diet-restricted rats received 40% less food compared to body-weight-matched AL rats consumed during the previous day. All rats were fed commercial rat diet (Table 1) for 9 weeks. To minimise the variation in feeding patterns between the rats fed AL and DR, rats were fasted 12 h prior to the collection of tissues.

At the end of the 9th week, the rats were killed under general anaesthesia. Testes were removed, and cauda epididymis was excised and placed in a 35-mm dish containing Dulbecco's phosphate-buffered saline. Each cauda epididymis was dissected with fine scissors, and spermatozoa were allowed to swim out for 10 min at 22 °C. The sperm suspension was gently drawn into a plastic Samco transfer pipette and placed in 5-ml tubes for further examinations. The testes were washed and stored at −80 °C until analysed. Thereafter, they were resuspended in the cold medium and homogenised using teflon/glass homogeniser.

Assay

Oxidative and antioxidative parameters were measured in the tissue samples using the Aeroset automated analyser (Abbott, IL, USA) and spectrophotometer (Cecil 3000, Cambridge, UK). Lipid hydroperoxide level (LOOH) was evaluated by the fluorometric method based on the reac-

Table 1 Contents of diet

Calculated contents of diet	%
Dry matter	88
Crude protein	24
Crude fibre	6
Fat	5
Ash	8
Calcium	1–2.8
Phosphorus	0.9
Vitamin premix[a]	
Metabolisable energy (kcal kg^{-1})	2.7

[a]The composition of vitamins per kilogram: Vit A, 14.000 IU; Carotene, 2.1 mg; Vit D_3, 2.500 IU; Vit E, 40 IU; Vit K_3, 1.2 mg; Vit B_1, 15 mg; Vit B_2, 5 mg; Vit B_6, 5 mg; Vit B_{12}, 0.05 mg; pantothenic acid, 20 mg; folic acid, 7 mg; biotin, 0.3 mg.

tion between malondialdehyde (MDA) and thiobarbituric acid (Conti *et al.*, 1991).

Total antioxidant status (TAS) (Erel, 2004) and total oxidant status (TOS) (Erel, 2005) were measured using an automated colorimetric measurement method. The per cent ratio of the TOS level to the TAS level was accepted as oxidative stress index (OSI), an indicator of the degree of oxidative stress. The unit of TAS was converted to μmol Eq. g^{-1} protein, and the OSI value was calculated using following formula: OSI = [(TOS, μmol H_2O_2 Eq. g^{-1} protein)/(TAS, μmol Trolox Eq. g^{-1} protein)].

Total free sulfhydryl groups (SH) of tissue were assayed according to the method of Hu *et al.* (1993). Ceruloplasmin (CP) activity was measured using method of Erel (1998) based on the enzymatic oxidation of ferrous ion to ferric ion. Paraoxonase (PON) activity was measured using paraoxon substrate. The rate of paraoxon hydrolysis was measured by monitoring the increase of absorbance at 412 nm (Eckerson *et al.*, 1983). Arylesterase (ARE) activity was analysed according to method of Haagen & Brock (1992).

Evaluation of sperm parameters

The sperm concentration was determined using Thoma haemocytometer (Smith & Mayer, 1955). The percentage of motile spermatozoa was determined using a phase-contrast microscope (BX-51; Olympus Tokyo, Japan) equipped with a heated (37 °C) stage.

Sperm plasma membrane integrity

Plasma membrane integrity was determined using propidium iodide and SYBR14 (FertiLight; Molecular Probes, Eugene, OR, USA). Treated and control sperm samples were incubated with SYBR14 and propidium iodide at 37 °C for 10 min. After staining, 100 spermatozoa were evaluated under epifluorescence microscopy (BX-51 Olympus Epifluorescent Microscope with U-51009 dual filter set). The sperm samples were classified into two groups according to the images of stained sperm head; a green fluorescence represents live intact cell membranes, whereas the red fluorescence represents damaged cell membranes or dead spermatozoon (Varisli *et al.*, 2013).

Sperm acrosomal integrity

Sperm acrosomal integrity was assessed using Arachis hypogaea, FITC-PNA conjugate (L7381 FITC-PNA; Sigma-Aldrich Co., St. Louis, MO, USA). Sperm specimens were incubated in the dark with FITC-PNA at 37 °C for 20 min and analysed under epifluorescence microscope. In each treatment, about 100 spermatozoa were counted to determine the proportion of spermatozoon with intact acrosomes. The images of stained sperm samples were classified into two groups according to the fluorescence of acrosomal region; intensive or moderate bright green fluorescence was considered nonintact or damaged, whereas weak or no fluorescence was regarded as acrosome intact (Nagy *et al.*, 2003; Coyan *et al.*, 2011).

Abnormal sperm assessment

Abnormal rat spermatozoon was assessed by the modified method of Wells & Awa (1970). Briefly, three drops of each sample were added to tubes containing Hancock solution. One drop of this mixture was put on a slide and covered with a cover slip. The percentage of total sperm abnormality was determined by counting 100 spermatozoa under phase-contrast microscopy.

Testosterone assay

Intratesticular testosterone level was measured using the electrochemiluminescence immunoassay (ECLIA) kit obtained from Roche Diagnostics GmbH, D-68298 Mannheim, Germany. Before the measurement, testosterone was extracted from homogenised testicular samples by twice in five volumes of diethyl ether. The organic phase was collected by centrifugation, and diethyl ether was evaporated in 35 °C water bath for 5–6 h. The extract was resuspended in an adequate volume of buffer from the kit. Interassay and intra-assay coefficients of variation (CV) were 10.3% and 6.4% for testosterone respectively.

Statistical analysis

The effects of temperature and DR, as well as their interaction on oxidative status of testis and sperm parameters, were analysed by two-way analysis of variance (ANOVA) using SPSS software 10.0 (SPSS Inc., Chicago, IL, USA). Multiple pairwise comparisons were evaluated by Tukey's multiple range test. Results were presented as means ± SD. Differences were considered significant at the $P < 0.05$ level.

Results

Long-term high ambient temperature did not increase oxidative (LOOH, TOS, OSI) and antioxidative (TAS) values in the rat testis ($P > 0.05$). However, some individual antioxidant parameters such as SH and CP were changed by high ambient temperature (Table 2). Long-term heat stress increased SH levels, while it decreased CP levels in the testis tissue. On the other hand, high temperature deteriorated the concentration, motility, volume,

Table 2 Effects of long-term dietary restriction and high temperature on oxidative status and sperm parameters in young rats (mean ± SD)

	RT (22 °C)		HT (38 °C)		Statistical analysis (*P* values)		
	AL (Group 1)	DR (Group 2)	AL (Group 3)	DR (Group 4)	Temp.	Feed	Interact
Oxidative status							
LOOH (μmol g^{-1} protein)	1.10 ± 0.09	1.02 ± 0.07	1.02 ± 0.07	1.05 ± 0.05	0.332	0.443	0.028
TOS (μmol H_2O_2 Eq. g^{-1} protein)	4.93 ± 0.32	4.69 ± 0.33	4.74 ± 0.43	4.95 ± 0.18	0.416	0.555	0.012
TAS (mmol Trolox Eq. g^{-1} protein)	0.24 ± 0.01	0.23 ± 0.01	0.23 ± 0.00	0.24 ± 0.01	0.206	0.313	0.007
OSI (Arbitrary units)	1.98 ± 0.12	1.93 ± 0.12	2.01 ± 0.24	2.05 ± 0.09	0.164	0.905	0.373
SH (mmol g^{-1} protein)	0.10 ± 0.03[a]	0.14 ± 0.01[b]	0.14 ± 0.01[b]	0.15 ± 0.00[b]	0.000	0.000	0.018
CP (U g^{-1} protein)	42.39 ± 2.55[a]	37.70 ± 1.33[b]	38.50 ± 1.92[b]	37.43 ± 0.84[b]	0.001	0.000	0.003
PON (U g^{-1} protein)	1.26 ± 0.55	1.66 ± 0.96	1.15 ± 0.50	0.95 ± 0.11	0.113	0.316	0.047
ARE (U g^{-1} protein)	1.50 ± 0.16[a]	1.26 ± 0.04[b]	1.47 ± 0.03[a]	1.29 ± 0.06[b]	0.958	0.000	0.280
Sperm parameters							
Motility (% motile spermatozoon)	76.67 ± 9.03[a]	75.98 ± 7.65[a]	58.88 ± 11.55[b]	60.34 ± 12.61[b]	0.000	0.907	0.746
Membrane integrity (%)	68.30 ± 9.91[a]	69.80 ± 9.55[a]	46.89 ± 7.34[b]	52.40 ± 10.68[b]	0.000	0.246	0.507
Acrosome integrity (%)	86.30 ± 3.12[a]	87.20 ± 6.76[a]	69.56 ± 7.05[b]	72.80 ± 10.39[b]	0.000	0.375	0.615
Concentration (spermatozoon × 10^6 ml^{-1})	23.60 ± 8.09[a]	20.96 ± 6.34[ab]	15.81 ± 5.70[b]	15.11 ± 7.94[b]	0.004	0.462	0.668
Abnormal spermatozoon (%)	23.60 ± 8.98[a]	19.20 ± 6.39[a]	45.00 ± 21.57[b]	52.30 ± 6.71[b]	0.000	0.717	0.150
Testicular testosterone (ng g $testis^{-1}$)	92.49 ± 16.58[a]	106.13 ± 45.24[a]	105.14 ± 32.14[a]	30.63 ± 13.03[b]	0.002	0.001	0.001
Testis weight							
Body weight (g)	343.60 ± 10.50[a]	275.90 ± 5.13[b]	281.89 ± 7.62[b]	247.20 ± 8.14[c]	0.000	0.000	0.000
Testis wt (g)	1.71 ± 0.11[a]	1.45 ± 0.12[b]	1.43 ± 0.09[b]	1.26 ± 0.08[c]	0.000	0.000	0.000
Testis wt/body wt (%)	0.49 ± 0.02	0.52 ± 0.03	0.50 ± 0.03	0.51 ± 0.02	0.555	0.512	0.612

a, b: means with different superscripts within a row are significantly different ($P < 0.05$).
RT, room temp.; HT, high temp.; DR, dietary restriction; AL, *ad libitum*; LOOH, lipid hydroperoxide; TOS, total oxidant status; TAS, total antioxidant status; OSI, oxidative stress index; SH, total free sulfhydryl groups; CP, ceruloplasmin; PON, paraoxonase activity; ARE, arylesterase activity.

and acrosome and membrane integrity of epididymal spermatozoon and increased abnormal sperm count ($P < 0.05$). Moreover, heat stress decreased body and testis weight ($P < 0.05$), while it did not affect ratio of testis/body weight. In addition, DR did not change oxidative or antioxidative parameters ($P > 0.05$) except from SH, CP and ARE activities ($P < 0.05$) in the rat testis. Dietary restriction increased SH levels, while it decreased CP activity in the rats housed at room temperature ($P < 0.05$). Dietary restriction application resulted in a decrease in the activity of ARE in both room and high temperature ($P < 0.05$). On the other hand, it was observed that DR not affected any sperm values ($P > 0.05$), whereas it decreased both body weight and testis weight ($P < 0.05$). Intratesticular testosterone level was only decreased in the dietary-restricted rats exposed to high temperature (Group 4).

Discussion

In the current study, we examined the protective effects of DR on testicular oxidative stress and sperm parameters in the young rats exposed to long-term heat stress. Reactive oxygen species generated by high temperature involves in the pathophysiologies of male infertility. Oxidative stress can be evaluated by both individual oxidant components such as MDA, F_2-isoprostanes separately, and total oxidant status, which is the cumulative action of all the oxidants present in tissues. The evaluation of total oxidative status can provide an integrated parameter rather than the simple sum of measurable oxidant (Celi, 2011). Similarly, antioxidant status can also be evaluated by both individual antioxidant components such as vitamin A, E, thiols, and total antioxidant status (TAS), which is the cumulative action of all the antioxidants in tissues. Furthermore, the ratio of TAS/TOS score has been defined as global oxidative stress index (OSI), reflecting both oxidative and antioxidant counterparts. This minimises the variability of the individual parameters of oxidative stress (TAS or TOS alone) and therefore constitutes a more powerful parameter (Verit *et al.*, 2006). In our study, TAS, TOS, OSI, and some oxidant and antioxidant parameters were measured separately.

Previous studies have reported that high-temperature exposure induced an increase on MDA level in the testis tissue (Kanter *et al.*, 2011) and peroxide levels in cell

culture of rat testis (Ikeda *et al.*, 1999). As for our study, heat stress was not observed to increase both LOOH and TOS, OSI values ($P > 0.05$) in the testis tissue, contrary to what we expected (Table 2). This result may be explained by a number of reasons. In animals, some physiological adaptations could occur to protect essential cell functions against increased temperature and to permit a rapid recovery from heat stress. The adaptive response to heat stress depends on animals' species, ageing and the duration of higher temperature (Ando *et al.*, 1997). The effect of heat on reproductive function also depends on the age of the male during exposure. Kurowicka *et al.* (2007) reported that immature male rats could be affected differently by high temperatures compared with adults. In the current study, the rats were young and exposed to long-term (9-week) high temperature. However, the rats in the research of Kanter *et al.* (2011) were exposed to short-term heat stress. Continuous exposure to high temperature for 3 weeks might increase the development of adaptive responses to oxidative stress, which cause physiological heat acclimatization. For example, Sod-Moriah *et al.* (1974) exposed heat-acclimatised for 3-month rats to an ambient temperature of 35 °C and observed a difference of 1.3 °C between testis of control (35 °C) and heat-acclimatised (36.3 °C) rat. In the light of these studies, ambient temperatures may not affect significantly the scrotal temperature in the heat-acclimatised animal.

Although no significant differences were observed on TAS values in the rats exposed to heat stress, different activity patterns were observed on individual antioxidant parameters in the current study (Table 2). For instance, the level of SH was increased, while CP activity was decreased by heat stress in the testis tissue ($P < 0.05$). Kasanuma *et al.* (1998) also reported that testicular-reduced glutathione concentration of rats exposed to heat stress tended to be higher than that of control rats. Paraoxonase has three known enzymatic molecules, PON1, arylesterase (ARE) and dyazoxonase. Paraoxonase-1 hydrolyses organophosphates such as paraoxon, arylesterase hydrolyses aromatic esters such as phenylacetate. Thus, both PON-1 and ARE as a single enzyme can decrease the accumulation of lipid peroxidation products (Erdem *et al.*, 2010). The activities of PON and ARE were found to be unchanged depending on heat stress ($P > 0.05$). These results suggest that the different regulation of individual antioxidant activities might be involved in the testis tissue of rat exposed to heat stress, although its exact mechanism is not known.

Previous studies have reported that DR can result in lower oxidative stress levels in various organs of different animal species (Xia *et al.*, 1995). On the contrary, DR did not decrease individual (LOOH) or cumulative oxidant parameters (TOS, OSI) in the testis of rats on both room temperature (22 °C) and high temperature (38 °C) in our study. Similarly, Ugochukwu *et al.* (2004) reported that 40% of DR application for 9 weeks has not produced any significant effect on the level of LOOH and some antioxidant enzymes activities in 3-month-old male Wistar rats. Dietary restriction resulted in a significant increase in testicular SH concentrations ($P < 0.05$) when compared to AL group in room temperature (Table 2). Higher testicular SH concentrations following long-time DR, which is possibly the result of the adaptation to lower dietary intake of SH precursors, could enhance resistance to major oxidative stressors. However, the effect of DR on SH content in testis was not observed on rats exposed to heat stress ($P > 0.05$). Paraoxonase and ARE activities have different patterns in response to DR. Long-term DR decreased testicular ARE activities significantly in both temperatures ($P < 0.05$), while it did not change PON activity significantly in this study. This discrepancy between PON and ARE may be due to differences in the specific regulation of both enzymes (Thomas-Moya *et al.*, 2006). It has been reported that the effect of DR depends on many variables such as age and duration and degree of restriction (Gredilla *et al.*, 2004). The majority of the findings regarding the beneficial effects of DR on antioxidant system were obtained in aged animals (Wu *et al.*, 2003). Young animals have an effective redox buffering system that provides protection from oxidative damage in a stressful situation (Zhang *et al.*, 2003). In the present study, the insufficient improving effect of DR on TAS, TOS and OSI values in the Groups 2 and 4 rats might result from that diet–restricted rats were young. On the other hand, after period of adaptation, diet-restricted rats consume the same amount or more food per gram of body weight than AL counterpart (Masoro, 2005). It also suggested that if the conversion of calories to body mass is similar in rats fed under both AL and DR conditions, metabolic rate, oxygen consumption and oxidative stress might be also similar.

In this study, heat-stressed rats had lower testicular weight compared with the rats on room temperature ($P < 0.05$). However, the ratio of testis weight/body weight (%) was not changed by heat stress and DR (Table 2). The loss of testicular weight might be due to cell-mediated apoptosis and germ cell loss (Kwon *et al.*, 2004; Li *et al.*, 2013). The motility, volume, acrosome and membrane integrity of sperm were negatively affected by heat stress ($P < 0.05$). High testicular temperature results in a period of infertility in several species, followed by a gradual return to normality over a period of one to two spermatogenic cycles (Kanter *et al.*, 2011). Therefore, short term a reduction in spermatid and spermatozoa

numbers might result from a failure of spermatocytes to complete their maturation cycle (Gasinska & Hill, 1990). For example, the bull spermatogenesis takes about 61 days, and alterations in semen occur about 2 weeks after heat stress and do not return to normal until 8 weeks following the end of heat stress (Hansen, 2009). Because the rats were exposed to long-term high heat stress, spermatozoa may be not able to complete maturation cycle, so that sperm count was decreased and abnormal spermatozoon was increased in the current study. There may also be a relationship between decrease in testis weight and low sperm count. On the other hand, DR did not have any protective effects on the sperm values in both temperatures ($P > 0.05$). Similarly, Sitzmann *et al.* (2010) reported that moderate CR has no detrimental or ameliorating effect on sperm parameters in young *rhesus macaques*. Furthermore, the means of sperm parameters in both AL and DR groups in room temperature (Groups 1 and 2) were within the normal ranges for Sprague–Dawley rats (Agca & Critser, 2005; Varisli *et al.*, 2013). In other words, DR failed to ameliorate abnormal sperm characteristics resulting from high ambient temperature.

In the current study, neither high temperature nor DR did change intratesticular testosterone levels. These results are consistent with the previous studies, in which DR did not affect plasma testosterone levels in monkey (Sitzmann *et al.*, 2010) and rats (Rehm *et al.*, 2008). However, intratesticular testosterone levels significantly decreased ($P < 0.05$) in the dietary-restricted rats exposed to high temperature (Group 4). This result may be attributed to nutritional impairment. For example, consuming feed of the rats exposed to high temperature (Group 3) decreased (13.04 ± 0.27 g day^{-1}) due to heat stress compared with Group 1 (19.12 ± 0.29 g day^{-1}) in the present study. On the other hand, the dietary-restricted rats exposed to high temperature (Group 4) were fed (8.35 ± 0.05 g day^{-1}) with 60% of the diet consumed by Group 3. Therefore, DR might result in severely food restriction in Group 4. In such circumstances, rats may be able to divert energy towards more critical functions of life maintenance. Similarly, Carvalho *et al.* (2009) reported that the testosterone decreased in the severely food-restricted rabbits.

In conclusion, long-term testicular heat stress does not affect global oxidant and antioxidant parameters, while it impaired all sperm values in the young rats. Furthermore, DR did not improve oxidative status and sperm quality in the young rats exposed to long-term high temperature.

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