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Full Length Research Paper

Effects of dietary vitamin E on daily intake, serum testosterone and epididymal sperm quality in Sprague-Dawley rats subjected to heat stress

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This study examined the effects of vitamin E on daily intake, serum testosterone levels, and epididymal sperm quality in heat stressed male rats. Rats were heat stressed (HS; 35°C) or housed at room temperature (control; 24°C) for 20 days and offered one of the following dietary vitamin E concentrations: 42 (low), 242 (med- low), 2,042 (med-high), or 20,042 (high) IU/kg diet. On day 21, rats were euthanized. In rats offered a low vitamin E diet, serum testosterone was greater (P = 0.002) in controls than in HS rats on the same diet. However, serum testosterone did not differ (P > 0.201) between controls and HS rats offered other dietary vitamin E levels. Epididymal sperm motility was reduced (P = 0.002) in HS rats compared to controls independent of vitamin E level, while sperm cell concentration was reduced (P = 0.010) in HS rats offered med-high dietary vitamin E compared to controls of the same diet. Sperm cell morphology did not differ between HS and control rats (P = 0.183). No sperm trait mentioned above was affected by dietary vitamin E level (P > 0.170). Testicular weight (P = 0.005) was reduced in HS rats, but not affected (P > 0.190) by vitamin E. Data indicate testicular mass loss and mild adverse effects on some components of male fertility due to heat stress However, these effects were inconsistent and were not relieved by increased dietary vitamin E concentration. Interestingly, no organ lesions were observed in rats consuming extreme amounts of vitamin E and vitamin E concentration did not influence intake or weight gain.

Key words: Epididymal sperm collection, heat stress, sperm quality, vitamin E.

INTRODUCTION

Subfertility in males exposed to heat stress is well-documented in sheep (Alliston, 1976) and other mammals (Setchell, 2006). In general, ambient temperatures that surpass upper limits of thermo-neutrality result in reduced fertility, even if limits are only marginally exceeded (Paul et al., 2008). Subfertility that is secondary to heat stress is characterized by testicular atrophy (Yin et al., 1997) reduced spermatogenic yields (Jannes et al., 1998), reduced sperm motility (Perez-Crespo et al., 2007) and increased incidence of abnormal sperm cells (Ax et al., 1987). However, previous authors have also noted unpredictability in response to heat stress, as well as high interspecies variability (Alliston, 1976; Setchell, 2006). Although much is known about effects of heat stress on testicular physiology, effects of heat stress on reproductive endocrinology in males is poorly understood. Ren et al. (2006) reported that testosterone in male rats is initially reduced by elevated testicular temperature; however, concentrations recover relatively quickly. Others reported that heat stress reduces steroidogenic efficiency in Leydig cells, but that LH secretion in these heat-stressed animals is increased proportionately, resulting in little or no net change in circulating testosterone (Demura et al., 1987; Setchell et al., 2001). Additionally, Lue et al.

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(2000) observed reduced effectiveness in the ability of circulating testosterone to stimulate other cells, which could lead to further increases in steroidogenesis. Because heat stress is known to increase oxidative stress (Mujahid et al., 2005; Marchetti et al., 2006), which in turn increases consumption of antioxidants, supple-mentation of vitamin E, a well-characterized antioxidant, could potentially attenuate adverse effects of heat stress on testicular physiology and reproductive endocrinology. Although previous research with vitamin E and fertility is somewhat inconsistent (Setchell, 2006; Marin-Guzman et al., 2000), vitamin E has been demonstrated to effectively reduce adverse effects on fertility by other forms of oxidative stress elicitation (Richards et al., 1999; El- Demerdash et al., 2004; Krishnamoorthy et al., 2007). The dietary level of vitamin E, if any, at which acute toxicity occurs is unclear in rats, although vitamin E appears to be relatively non-toxic in humans at relatively high levels (Stampfer et al., 1993). The goals of this study were to investigate effects of prolonged, daily heat stress on the reproductive ability of mature, male rats and to investigate the effects, if any, of increased levels of dietary vitamin E on such effects. The consequences of offering massive amounts of dietary vita- of dietary vitamin E on acute organ health, average daily gain and daily dietary intake were also examined.

MATERIALS AND METHODS

Experimental procedure

All procedures involving animals were approved by the New Mexico State University Institutional Animal Care and Use Committee. Forty-eight mature, genetically- related male Sprague-Dawley rats (18 months of age; 699 ± 12 g) provided by the New Mexico State University Small Animal Care facility were housed in individual 15 x 15 x 30 cm plexiglass cages and offered free access to clean drinking water. Absorbent bedding (approximately 2 cm) was placed at the bottom of the cage and changed every 5 days. Automatic lights were used to provide 12 h of light (consecutive) and 12 h of dark each day. Following a 10- day adjustment period that included experimental diets, rats were randomly assigned to one of two test groups: i.) heat stressed (HS; 35° ± 1° C for 6 h daily for 20 days, 24° ± 1°C for 18 h; n = 24 or ii.) thermoneutral ambient temperature (control; 24° ± 1°C, constant temperature for 20 days; n = 24) as described by Romanovsky et al. (2002). Additionally, rats in each temperature treatment were offered varying levels of dietary vitamin E. Rats exposed to HS were offered identical basal diets (Table 1) containing 1 of 4 levels of vitamin E: 42 (low), 242 (med-low), 2,042 (med-high), or 20,042 (high) IU vitamin E/kg. Vitamin E levels contained within low and med-low diets were consistent with commercial maintenance and breeding-animal diets, respectively, for laboratory rats. Vitamin E added to med- high and high diets was 10 × and 100 ×, respectively, of that added to med-low diet in an effort to examine possible toxicity. Diets were offered *ad libitum* and intake was recorded daily at the conclusion of the heat-stress period. Each control rat was paired randomly to a HS rat and was offered the type and quantity of diet consumed by its HS counterpart. Body weights of all rats were recorded at day 0, 10 and 20. On day 21, rats were anes-thetized with isoflourine gas (IsoFlo, Abbott Animal Health, Abbott Park, IL) and an epididymal sperm sample was obtained. Rats were then euthanized via heart-puncture exsanguinations followed by rapid guillotine decapitation. Testes, livers, and hearts were weighed at necropsy, and livers, hearts, kidneys and gastrointestinal tracts were inspected for abnormality. A single rat (control, high) died before the conclusion of the study and two rats (control, med-high; HS, low) developed tumors and were removed from the study.

Table 1. Nutrient analysis (dry matter) of basal diet offered to male Sprague-Dawley rats experiencing heat stress for 20 days.

Nutrient	Amount
Crude protein, %	23.0
Ether extract, %	4.5
Crude fiber, %	6.0
Neutral detergent fiber, %	15.6
Acid detergent fiber, %	6.7
Nitrogen-free extract, %	48.7
Total digestible nutrients, %	76.0
Metabolizable energy, kcal/g	3.02
Calcium, %	0.95
Phosphorous, %	0.66
Potassium, %	1.18
Magnesium, %	0.21
Selenium, ppm	0.30
Vitamin A, IU/g	15.0
Vitamin D, IU/g	4.5
Vitamin E, IU/kg	42.0
Ascorbic acid, mg/g	0.0

[1]Vitamin E was added to base diet at a concentration of 0 (low), 200 (med-low), 2,000 (med-high), or 20,000 (high) IU/kg feed.
[2]Diet was fed free choice to heat-stressed rats. Control rats were pair-fed for diet type and quantity.

Sample collection and analysis

Serum testosterone concentration

While under deep anesthesia, blood was collected from each rat via heart puncture using an 18-gauge hypodermic needle. The needle was inserted into the chest cavity parallel with the center of the forelimb just left of the sternum until blood could be observed pulsating into the base of the needle. Blood was extracted with a 6 ml syringe and transferred to a 10 ml vacuum tube (Corvac serum-separator, Kendall Health Care, St. Louis, MO). Samples were left at room temperature for 30 - 60 min and then centrifuged (1,500 x g at 4°C, 15 min). Serum was collected into plastic vials and stored at - 80°C. Serum testosterone was quantified by solid phase RIA using components of a commercial kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) with modifications described by Richards et al. (1999). To verify assay performance, a pool of serum was obtained as previously described from a mature female Sprague-Dawley rat. This pool was used to prepare spiked samples containing 2.5, 5.0, 10.0, and 15.0 ng of testosterone/ml of serum (Sigma-Aldrich Co., St. Louis, MO). When 50 µl of these samples were assayed, 2.1 (CV = 9.3, 81% recovery), 4.6 (CV = 2.1, 90% recovery), 8.9 (CV = 9.2, 88% recovery), and 17.3 (CV = 2.4, 114% recovery) ng/ml were obtained, respectively. Addition of 25, 50, and 100 µl of rat serum resulted in displacement parallel to parallel to the standard curve. Specific binding of tracer to the

Table 2. Serum testosterone concentration (ng/ml) at day 21 in male Sprague-Dawley rats fed 1 of 4 dietary levels of vitamin E and exposed to heat stress for 20 days.[1]

Vitamin E level	Temperature		SE[4]	*P* - value
	Control[2]	Heat stress[3]		
Low[5]	1.9	0.1	0.32	< 0.001
Med-low[6]	0.3	0.8	0.32	0.201
Med-high[7]	0.4	0.2	0.36	0.682
High[8]	0.3	0.4	0.32	0.771

[1]A temperature x vitamin E level interaction was observed (*P* = 0.006).[2] 24° ± 1° C, constant temperature. Rats were pair-fed for type and quantity of diet with a randomly assigned heat-stressed rat.
[3] 35° ± 1° C for 6 h daily, 24° ± 1° C for 18 h. Rats were offered free choice access to diet. [4] Standard error (n = 6).[5] 42 IU vitamin E/kg diet.[6] 242 IU vitamin E/kg diet.[7] 2,042 IU vitamin E/kg diet.[8] 20,042 IU vitamin E/kg diet.

antiserum was 60.5 %. Sensitivity of the assay (95 % of maximum binding) was 0.02 ng/ml. Samples were quantified in a single assay (CV = 12.9%) and duplicate 50 µl aliquots were used to quantify testosterone in all serum samples.

Epididymal sperm cell motility, concentration and morphology

Epididymal sperm samples were collected after rats were put under deep anesthesia but before exsanguinations. Rats were placed in a supine position and hair was removed from the scrotum with scissors. Beginning at the base of the scrotum, a 1 cm vertical incision was made through the scrotum and tunica, and the entire testicle was exposed. The vas deferens was isolated just above the cauda epididymus. Two 2 cm bulldog clamps (Roboz Surgical Instrument Co., Gaithersburg, MD) were used to block blood vessels above the isolated section and below the epididymus. Soft-tissue scissors (Roboz Surgical Instrument Co.) were used to sever the vas deferens at the point of connection to the epididymus. Sperm forced from the epididymus with gentle pressure applied to the lower section of the epididymus was collected into a 70 µl capillary tube (Fisher Scientific, Pittsburgh, PA). A small amount of sperm was immediately transferred to a heated (26°C) glass microscope slide and sperm motility percentage was estimated as described by Sorensen (1979) using a light microscope (Swift Microscope World, Carlsbad, CA). Motility determinations (estimated percentage of forwardly progressive motile sperm cells) were made at 10 different locations on the slide and average motility percentages were recorded (n = 6 or n = 45). A volume of 5.0 µl of sperm was diluted into 150 µl of saline and 0.1 ml of diluted sample was placed onto a hemacytometer (Hausser Scientific, Horsham, PA) for determination of sperm cell concentration (n = 6 or n = 45; Sorensen, 1979). Lastly, a small volume of diluted sample was placed on a glass microscope slide and sperm cell morphology was determined by evaluation of 10 groups of 10 individual cells in various locations on the slide (n = 6 or n = 45; Sorenen, 1979).

Statistical analysis

Daily dietary intake data were treated as a split plot design with temperature treatment in the main plot and day and temperature × day interaction included in the sub-plot. Intake data were analyzed using the mixed procedure of SAS (SAS Inst., Inc., Cary, NC) with repeated measures function. Serum testosterone concentration, sperm cell motility, concentration, and morphology, and total testicular weight, heart weight, and liver weight were analyzed as a completely randomized design with a 2 × 4 factorial treatment structure (GLM procedure of SAS; n = 6/cell). Where temperature x vitamin E level interactions was observed, temperature effects were examined within vitamin E level and vitamin E effects were examined within temperature treatment. Total testicular weight, heart weight, and liver weight were expressed as a percentage of final body weight. Rat was considered the experimental unit for all comparisons. For instance where an observed value deviated more than 4 standard errors from the mean of its respective cell, the value was considered an outlier and omitted from statistical analysis.

RESULTS AND DISCUSSION

Where temperature × vitamin E interactions level were observed (P = 0.006) for serum testosterone concentration. In rats offered a low vitamin E diet, serum testosterone concentration was greater (P < 0.001) in control rats when compared to HS rats (Table 2). However, serum testosterone concentration did not differ significantly (P > 0.20) between HS and control rats offered med-low, med-high, or high vitamin E diets. Similar irregularities in testosterone response to heat stress have been previously reported. For instance, Demura et al. (1987) demonstrated that reduced activity of testosterone-producing Leydig cells during heat stress is offset by increased secretion of LH, resulting in no net change in circulating testosterone concentration. Additionally, Ren et al. (2006) reported an initial decrease in testosterone was followed by full recovery, while Lue et al. (2000) reported a decreased stimulatory ability of testosterone on other cells during periods of heat stress that could further promote increased magnitude of circulating testosterone. Although previous research has indicated that vitamin E may preserve Leydig cell function during noxious stress (Richards et al., 1999), it is unclear from serum testosterone concentrations in the present study if vitamin E elicits similar effects in animals exposed to heat stress. Total testicular weight (P = 0.021; Table 3) and total testicular weight/body weight (P = 0.005) were reduced in HS rats compared to controls.

Table 3. Total testicular weight (g) and total testicular weight/body weight (%) at day 21 in male Sprague-Dawley rats fed 1 of 4 dietary levels of vitamin E and exposed to heat stress for 20 days.[1]

Item	Temperature		SE[2]	Vitamin E level[3]				SE[2]
	Contro[4]	Heat stress[5]		Low	Med-low	Med-high	High	
Total testicular weight	7.28[a]	6.58[b]	0.285	6.57	6.66	7.14	7.33	**0.286**
Total testicular weight : Body weight	1.1[a]	0.9[b]	0.05	1.0	1.0	1.0	1.1	**0.05**

[1] No temperature x vitamin E level interaction was observed for total testicular weight (P = 0.648) or total testicular weight : body weight (P = 0.516).
[2] Standard error (n = 24/cell and 12/cell for temperature and vitamin E level, respectively).
[3] Low, med-low, med-high, and high contain 42, 242, 2,042, and 20,042 IU/kg diet, respectively.
[4] 24° ± 1° C, constant temperature. Rats were pair-fed for type and quantity of diet with a randomly assigned heat-stressed rat.
[5] 35° ± 1° C for 6 h daily, 24° ± 1° C for 18 h. Rats were offered free choice access to diet.
[a,b] Row values within main effect treatment with different superscripts differ (P < 0.021).

Table 4. Epididymal sperm cell parameters at day 21 in male Sprague-Dawley rats fed 1 of 4 dietary levels of vitamin E and exposed to heat stress for 20 days.[1]

Item	Vitamin E level	Temperature		SE[2]	*P* - value
		Control[3]	Heat stress[4]		
Motility, %	All	46	24	4.6	0.002
Concentration, x 10^3	Low[5]	234.5	196.0	26.6	0.282
	Med-low[5]	220.5	197.8	23.8	0.504
	Med-high[5]	260.1	176.8	23.8	0.010
	High[5]	195.5	248.5	23.8	0.170
Morphology, %	All	68	62	3.1	0.183

[1] A temperature x vitamin E level interaction was observed for concentration (P = 0.050), but not for motility (P = 0.069) or morphology (P = 0.858).
[2] Standard error (n = 24, 6, and 24 for motility, concentration, and morphology, respectively).
[3] 24° ± 1° C, constant temperature. Rats were pair-fed for type and quantity of diet with a randomly assigned heat-stressed rat.
[4] 35° ± 1° C for 6 h daily, 24° ± 1° C for 18 h. Rats were offered diet free choice.
[5] Low, med-low, med-high, and high diets contained 42, 242, 2,042, and 20,042 IU vitamin E/kg diet, respectively.

Testicular mass loss observed in the present study reflects observations in previous work, as several groups have demonstrated loss of testicular weight in heat-stressed adult mice (Yin et al., 1997; Jannes et al., 1998; Paul et al., 2008). Additionally, *in vitro* studies indicate that loss of testicular mass may be due to cell-mediated apoptosis rather than necrosis (Kwon et al., 2004). Although epididymal rats controls, motility did sperm motility was reduced (P = 0.002) in exposed to heat stress compared to not differ (P = 0.429) among vitamin E levels (Table 4). Reduction of motility by heat stress in the current study supports similar results from previous work. Ax et al. (1987) demonstrated that sperm collected from heat-stressed mature bulls exhibited reduced motility, which correlated to reduced binding affinity for proteins such as hydrogen-3 heparin. Jannes et al. (1998) reported similar depression of sperm motility in mice. Impairment of sperm cells by heat stress is believed to occur primarily in development stages (Banks et al., 2005) and although mature sperm cells appear to be far less vulnerable to damage (Perez-Crespo et al., 2007), sperm collection in the present study was conducted after 20 days of heat stress.

Thus semen specimens collected in the present study should have contained a mix of sperm cells that represented all stages of spermatogenesis, as the spermatogenic cycle of the rat lasts 19 days (Love and Kenney, 1999). An interaction was observed (P = 0.050) between temperature and vitamin E level for epididymal Sperm cell concentration. Epididymal sperm cell concentration (Table 4) was reduced (P = 0.010) by heat stress in rats offered med-high vitamin E, but did not differ (P > 0.170) between temperatures in rats offered low, med-low, or high vitamin E. Although heat stress has been shown to reduce sperm concentration, this response appears to exhibit a great deal of animal to animal variability (Alliston, 1979). Differences between HS and control rats offered med-high vitamin E support previous findings (Jannes et al., 1998; Perez-Crespo et al., 2007), but inconsistent responses across vitamin E levels may be indicative of large natural variation in individual responses to heat stress. Surprisingly, epididymal sperm morphology did not differ due to temperature (P = 0.183) or vitamin E level (P = 0.858). This finding is not consis-

Table 5. Liver and heart weights (% of body weight) at day 21 in male Sprague-Dawley rats fed 1 of 4 dietary levels of vitamin E and exposed to heat stress for 20 days.[1]

Item	Temperature		SE[2]	Vitamin E level[3]				SE[2]
	Control[4]	Heat stress[5]		Low	Med-low	Med-high	High	
Liver weight : body weight	3.23	3.26	0.158	3.38	3.35	3.18	3.08	0.211
Heart weight : body weight	0.29	0.31	0.014	0.32	0.30	0.30	0.29	0.016

[1] No temperature x vitamin E level interaction was observed liver weight: body weight (P = 0.927) or heart weight: body weight (P = 0.776).
[2] Standard error (n = 24/cell and 12/cell for temperature and vitamin E level, respectively).
[3] Low, med-low, med-high, and high contain 42, 242, 2,042, and 20,042 IU/kg diet, respectively.
[4] 24° ± 1°C, constant temperature. Rats were pair-fed for type and quantity of diet with a randomly assigned heat-stressed rat.
[5] 35° ± 1° C for 6 h daily, 24° ± 1° C for 18 h. Rats were offered free choice access to diet.

tent with previous findings which reported that heat stress treatment reduced the percentage of normal sperm (Ax et al., 1987; Love and Kenney, 1999). Although vitamin E has been shown to reduce adverse effects on sperm quality characteristics in animals facing other forms of oxidative stress (El-Demerdash et al., 2005; Krishnamoorthy et al., 2007), no such benefit was obtained in the current study by increasing dietary vitamin E in HS rats. Observations in the current study concerning sperm quality in control rats offered varying concentrations of vitamin E were expected, as Marin-Guzman et al. (2000) showed no vitamin E effects on sperm in the absence of stress.

Daily intake in HS rats did not differ (P = 0.701) among vitamin E levels in the current study. Although dietary intake has long been thought to be inversely related to ambient temperature (Brobeck, 1948), recent work with feedlot cattle demonstrated that animals fed free choice and exposed to interrupted heat stress do not necessarily exhibit depressed daily intake (Madar and Davis, 2004). Of note, daily intake differed (P = 0.007) among days, with greatest intake occurring on days 4, 5, and 6 and least intake occurring on day 1, the first day of heat stress.

Average daily gain over the 20-day experimental period did not differ between temperatures (P = 0.252) or among vitamin E levels (P = 0.947). Despite previous reports of reduction of body weight in animals experiencing heat stress (Kamal and Johnson, 1971), lack of differential average daily gain between HS and control rats in the current study is not surprising after considering similarities in dietary intake. Additionally, no difference in daily intake or average daily gain among vitamin E levels suggests that vitamin E did not influence palatability of feed or elicit adverse post-ingestive feedback even when present in the diet in extraordinary amounts. This compliments previous work in humans that indicates vitamin E is not toxic, even in large quantities (Stampfer et al., 1993). Liver weight/body weight (Table 5) did not differ between temperatures (P = 0.872) or among vitamin E levels. This finding provides further evidence that large amounts of dietary vitamin E were not noticeably toxic. Similarly, heart weight/body weight did not differ between temperatures (P = 0.441) or among vitamin E levels (P = 0.742) . Upon necropsy, no major lesions or abnormalities were observed in the liver, heart, kidney, or gastrointestinal tract in any of the rats.

Data collected from this study indicate that 6 h of daily heat stress for 20 days resulted in loss of testicular mass that was not corrected by increased consumption of vitamin E. Testicular atrophy in heat-stressed rats was accompanied by reduced serum testosterone concentration and epididymal sperm cell concentration in some rats, but these observations were not consistent through all dietary vitamin E levels. Irregularities in testosterone concentration and other reproductive traits may reflect large animal to animal variability in physiological response to heat stress that has been previously reported by Alliston (1976) and Setchell (2006). Heat stress reduced epididymal sperm motility in all rats, but vitamin E failed to restore motility at any offered level. Surprisingly, epididymal sperm cell morphology was not altered by heat stress in the current study, despite numerous previous studies to the contrary. Average daily gain and daily dietary intake data as well as organ weights and appearances suggest that vitamin E is neither unpalatable nor acutely toxic, even when consumed in exceedingly large quantities. Results from this study demonstrate adverse effects of heat stress on reproductive traits in male rats, but also appear to offer further evidence of large animal-to-animal variability in these effects. There were, however, no observations from this study that suggests vitamin E influences physiological response to daily heat stress in male rats.

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REFERENCES

Alliston CW (1976). Effects of heat on reproduct. in sheep. Prog. Biometeorol. Div. B. Prog. Anim.Biometeorol. 1(1): 381-386.
Ax RL, Gilbert GR, Shook GE (1987). Sperm in poor quality semen

from bulls during heat stress have a lower affinity for binding hydrogen-3 heparin. J. Dairy Sci. 70: 195-200.
Banks S, King SA, Irvine DS, Saunders PTK (2005). Impact of a mild scrotal heat stress on DNA integrity in murine spermatozoa. Reprod. 129: 505-514.
Brobeck, JR (1948). Food intake as a mechanism of temperature regulation. Yale J. Biol. Med. 29: 545-552.
Demura RT Suzuki, Nakamura S, Komatsu H, Jibiki K, Odagiri E, Demura H, Shizume K (1987). Effect of uni- and bilateral cryptorchidism on testicular inhibination and testosterone secretion in rats. Endocrinol. Jpn. 34(6): 911-917.
El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH (2004). Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and -carotene. Food Chem. Toxicol. 42: 1563-1571.
Jannes P, Spiessens C, Van der Auwera I, Hooghe T D, Verhoeven G, Vanderschueren D (1998). Male subfertility induced by acute scrotal heating affects embryo quality in normal female mice. Hum. Reprod. 13(2): 372-375.
Kamal T H, Johnson H D (1971). Total body solids loss as a measure of a short-term heat stress in cattle. J. Anim. Sci. 32: 306-311.
Krishnamoorthy G, Venkatarman P, Arunkumar A, Vignesh RC, Aruldhas MM, Arunakaran J (2007). Ameliorative effect of vitamins (-tocopherol and ascorbic acid) on PCB induced oxidative stress in rat epididymal sperm. Reprod. Toxicol. 23: 239-245.
Kwon J, Wang Y L, Setsuie R, Sekiguchi S, Sato Y, Sakurai M, Noda M, Aoki S, YoshikawaY, Wada K (2004).Two closely related ubiquitin C-terminal hydrolase isozymes function as recriprocal modulators of germ cell apoptosis in cryptorchid testis. Am. J. Pathol. 165:1367-1374.
Love CC, Kenney RM (1999). Scrotal heat stress induces altered sperm chromatin structure associated with a decrease in protamine disulfide bonding in the stallion. Biol. Reprod. 60: 615-620.
Lue YA, Hikim PS, Wang C, Im M, Leung A, Swerdloff RS (2000). Testicular heat exposure enhances the suppression of spermatogenesis by testosterone in rats: The “two-hit” approach to male contraceptive development. Endocrinol. 141: 1414-1424.
Mader TL, Davis MS (2004). Effects of management strategies on reducing heat stress of feedlot cattle: Feed and water intake. J. Anim. Sci. 82: 3077-3087.
Marchetti MA, Weinberger, Murakami MY, Burhans WC, Huberman JA (2006). Production of reactive oxygen species in response to replication stress and inappropriate mitosis in fission yeast. J. Cell. Sci. 119:124-131.
Marin-Guzman J, Mahan DC, Whitmoyer R (2000). Effects of dietary selenium and vitamin E on the ultrastructure and ATP concentration of boar spermatozoa, and the efficacy of added sodium selenite in extended semen on sperm motility. J. Anim. Sci. 78: 1544-1550.
Mujahid A, Yoshiki Y, Akiba Y, Toyomizu M (2005). Superoxide radical production in chicken skeletal muscle induced by acute heat stress. Poultry Sci. 84: 307-314.
Paul CA, Murry A, Spears N, Saunders PTK (2008). A single, mild, transient scrotal heat stress causes DNA damage, subfertility, and impairs formation of blastocysts in mice. Reprod. 136: 73-84.
Perez-Crespo M, Pintado B, Gutierrez-Adan A (2007). Scrotal heat stress effects on sperm viability, sperm DNA integrity, and the offspring sex ratio in mice. Mol. Reprod. Develop. DOI 10.1002/mrd.20759.
Ren L, Medan MS, Ozu M, Li C, Wananabe G, Taya K (2006). Effects of experimental cryptorchidism on sperm motility and testicular endocrinology in adult male rats. J. Reprod. Dev. 52: 219-228.
Richards JB, Hallford DM, Duff GC (1999). Serum luteinizing hormone, testosterone, and thyroxine and growth responses of ram lambs fed locoweed (*Oxytropis sericea*) and treated with vitamin E/selenium. Theriogenol. 52:1055-1066.
Romanovsky AA, Ivanov AI, Shimansky YP (2002). Selected contribution: Ambient temperature for experiments in rats: a new method for determining the zone of thermoneutrality. J. Appl. Physiol. 92: 2667-2669.
Setchell B P (2006). The effects of heat on the testes of mammals. Anim. Reprod. 3: 81-91.
Setchell B P, Ploen L, Ritzen EM (2001). Reduction of long-term effects of local heating of the testis by treatment of rats with a GnRH agonist and an anti-androgen. J. Reprod. 122(2): 255-263.
Sorensen AM (1979). Reproductive laboratory: A laboratory manual for animal reproduction. 4th Ed. American Press. Boston, MA.
Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett W C(1993). Vitamin E consumption and the risk of coronary disease in women. N. Engl. J. Med. 328: 1444-1449.
Yin Y, Hawkins KL, DeWolf WC, Morgentaler A (1997). Heat stress causes testicular germ cell apoptosis in adult mice. J. Androl. 18(2): 159-165.