Effects of chronic heat stress on testicular structures, serum testosterone and cortisol concentrations in developing lambs

Aria Rasoolia, Mohammad Taha Jalali, Mohammad Nouri, Babak Mohammadian, Farid Barati

A Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran
B Department of Laboratory Science, Paramedical School, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
C Department of Pathobiological Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran

Abstract

The objective of this study was to investigate the effect of high and moderate summer ambient temperatures on testicular structures and endocrine profile of developing ram lambs. Twenty fall-born ram lambs were randomly divided into two groups: animals were kept outdoor (n = 10) under ambient temperature (31–50 °C) or maintained indoor (26–32 °C) from May to October 2007. Monthly serum testosterone and cortisol concentrations were compared between two groups throughout the experiment. The animals were slaughtered at the end of the study and their testes subjected to histopathology exam. The results showed that maximum outdoor ambient temperature was significantly higher than indoor. There was no difference between two groups on serum testosterone concentration. There was no effect on serum cortisol levels except in August and October. Histopathological examination revealed a severe testicular degeneration with significant germ line degeneration without any impact on somatic cells. In conclusion, direct exposure of developing lambs during non-breeding season impairs testicular germ cells without significant effect on testicular endocrine function.

Keywords: Heat stress, Developing lamb, Testicular structures, Testosterone, Cortisol

1. Introduction

It is well known that the required temperature for normal mammalian spermatogenesis is 2–4 °C less than internal body temperature (Pineda, 2003). Decreased male fertility in heat stressed bull (Nichi et al., 2006) and human (Jung and Schuppe, 2007) as well as impact of the high body temperature on testicular structures and function in cryptorchid testes (Barenton et al., 1982; Lunstra and Schanbacher, 1988; Ren et al., 2006) indicated the suppressive effects of heat stress on testicular functions (Johnson et al., 1969; Gomes et al., 1971; Wettemann and Desjardins, 1979; Sailer et al., 1997). In vitro and in vivo exposure of testis to high thermal stress impairs spermatogenesis by elimination of spermatogonial germ cells in the seminiferous tubules and degeneration of sertoli and leydig cells (Gomes et al., 1971) and reduces sperm fertility (Yaeram et al., 2006). However, there are conflicted data reported on the serum testosterone profile in heat exposed testes (Wettemann and Desjardins, 1979; Barenton et al., 1982; Lunstra and Schanbacher, 1988; Sidibe et al., 1992).

Sheep are seasonal breeder animal which are sexually inactive during the summer months (Pineda, 2003). There are some studies reported decreased spermatogenesis and testicular function during summer in this specie which is related to photoperiod (Gomes and Joyce, 1975; Lincoln, 1976; Schanbacher and Ford, 1976; Dufour et al., 1984; Rhim,...
et al., 1993). In some tropical regions ambient temperature rises up to more than 50 °C during summer months. This factor may increase the suppressive effect of photoperiod on testicular function in ram (Gomes et al., 1971). The objective of this study was to evaluate the effects of the summer heat stress on testicular structures and serum testosterone and cortisol concentrations of developing ram lambs.

2. Materials and methods

2.1. Experimental location

The experiment was conducted from 1 May to 30 October 2007 at the veterinary medicine hospital, Shahid Chamran University of Ahvaz at the Khuzestan province of Iran (latitude: 31° 20′N; longitude: 48° 41′E; altitude: 19 m). The region was considered to be arid environment with an annual rainfall of 168 mm and temperature of 25.9 °C with values daily ranging from 0 to 17 mm and 1.8–50.1 °C, respectively.

2.2. Experimental animals

Fall-born (September and November) ram lambs (n = 20; 19.9 ± 0.79 kg LW; 6.2 ± 1.12 months) subjected to indoor and outdoor conditions. All lambs received a ration comprised of wheat straw, Lucerne hay and concentrate. Water and minerals were available ad libitum for both groups.

2.3. Experimental design

The male lambs were randomly divided into two groups; one (n = 10; 18.7 ± 1.19 kg LW) subjected to outdoor conditions with a temperature range from 31 to 50 °C, and the other, which was kept indoors (n = 10; 21.02 ± 1.02 kg LW) with minimum and maximum temperature of 26 and 32 °C, respectively. The entire period of study lasted six months (from May to October 2007). Outdoor animals were kept in a conventional open shed under direct solar radiation and the indoor animals were maintained in a room equipped with an air conditioner. Both groups were exposed to same duration of natural photoperiod throughout the experiment.

2.4. Measurements

Daily maximum environmental temperatures were recorded for both groups. On the first day of each month, blood samples were collected from jugular vein, allowed to stand for 20 min and centrifuged at 3000 rpm for 10 min. Then serum was separated and stored at −20 °C till it was convenient to assay for testosterone and cortisol concentrations. At the end of the experiment all animals were slaughtered and testes were immediately removed, weighted by a mechanical balance, fixed in 10% formaldehyde for 48 h and subjected to histopathologic assessment.

2.5. Histopathologic procedures

The pieces of formaldehyde fixed tissues (1 × 1 × 1 cm³) dissected, dehydrated in an ethyl alcohol series and embedded in paraffin wax. Sections were cut at 5 μm thickness, stained with Hematoxylin and Eosin (H&E) and examined by light microscopy.

2.6. Hormone analysis

Serum concentrations of testosterone were quantified using LIAISON® Testosterone (310410) assay kit (DiaSorin Inc., USA). The procedure was based on direct, competitive, chemiluminescence immunoassay (CIA). Cross-reactivity was 2.8% with 5α-dihydrotestosterone. The intra- and interassay coefficient of variation (CV) for six assays were less than 8 and 10%, respectively.

Serum concentrations of cortisol were measured using LIAISON® cortisol assay kit (DiaSorin Inc., USA). The procedure was competitive luminometric assay based on Solid Phase Antigen Linked Technique (SPLT). In this procedure cortisol is used for the coating of the solid phase (magnetic particles). The tracer consists of highly specific monoclonal antibody, which is labeled with an isoluminal derivative. Maximum cross-reactivity was 50% with corticosterone. The intra- and interassay CV were less than 5.1% (20 assays) and 6.9% (59 assays), respectively.

2.7. Statistical analysis

Daily maximum environmental temperatures throughout the months of experiment as well as the main effect of environments (indoor vs. outdoor), months of experiment and their interaction on serum testosterone and cortisol concentrations were analyzed by least squares ANOVA using the General Linear Models procedure of SAS. Least squares were separated with pdiff analysis of SAS (1996). Mean weight of both testes (right and left) for each lamb were compared between two groups by t-test procedure of SAS (1996). Any correlation between testis weight and serum testosterone concentration of males at October was analyzed by Pearson correlation procedure of SAS. Data was expressed as LSmean ± S.E.M.

3. Results

Maximum outdoor (44.25 ± 0.15; 43–50 °C) ambient temperature was significantly higher than indoors (Fig. 1: 30.24 ± 0.16; 30–38 °C).

Serum testosterone concentration was not affected by direct exposure of animals to summer heat stress (P > 0.05)

![Fig. 1. The maximum ambient temperature during months of experiment for indoor and outdoor ram lambs (LSmeans ± S.E.M.).](#)
Table 1
Monthly serum testosterone and cortisol concentrations (LSmeans ± S.E.M.) in the developing lamb rams exposed to different environmental temperatures (outdoor: n = 10 and indoor: n = 10) during non-breeding season.

<table>
<thead>
<tr>
<th>Months</th>
<th>Testosterone (ng/ml)</th>
<th>Cortisol (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outdoor</td>
<td>Indoor</td>
</tr>
<tr>
<td>May</td>
<td>0.1 ± 0.55&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>0.6 ± 0.55&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>June</td>
<td>0.4 ± 0.39&lt;sup&gt;abA&lt;/sup&gt;</td>
<td>0.8 ± 0.35&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>July</td>
<td>0.6 ± 0.39&lt;sup&gt;abA&lt;/sup&gt;</td>
<td>1.2 ± 0.41&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>August</td>
<td>0.4 ± 0.39&lt;sup&gt;abA&lt;/sup&gt;</td>
<td>1.1 ± 0.39&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>September</td>
<td>1.4 ± 0.39&lt;sup&gt;bcA&lt;/sup&gt;</td>
<td>1.1 ± 0.37&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>October</td>
<td>2.2 ± 0.35&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>1.6 ± 0.35&lt;sup&gt;AA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with different superscripts within columns (<sup>abc</sup> for testosterone and <sup>def</sup> for cortisol) significantly differ P<0.05.
Values with different superscripts within rows (<sup>a</sup> for testosterone and <sup>AE</sup> for cortisol) significantly differ P<0.05.

Fig. 2. Microscopic views of normal [a (100×), b (40×)] and degenerated [c (40×), d (40×)] testes of animals exposed to different ambient temperatures (outdoors and indoors). Spermatogonia: (Spg), spermatocyte (Spc), spermatid (Spd), sertoli cell (St), leydig cells (Ld), multinucleated giant cells (Mgc) and vacouelated seminiferous tubules (Vst).

compare with indoor animals. However, it was slightly (P>0.05) higher in indoor (1.05 ± 0.17 ng/ml) than outdoor (0.84 ± 0.16 ng/ml) animals. Within both groups, monthly serum testosterone concentration increased (Table 1; P<0.05) while the animals were entering to the breeding season (September and October). There was no significant association between testis weight and the October serum testosterone concentration (r = 0.473, p = 0.074).

Mean testicular weights were significantly higher (P<0.05) in indoor (151.01 ± 20.9; 86.645–226.5 g) than outdoor (90.98 ± 17.29; 15.1–164.85 g) animals.

Histopathologic examination revealed a severe testicular degeneration mostly in the germ line cells without significant effect on somatic cells (leydig and sertoli cells). Vacouelation or disappearance of seminiferous tubules epithelium lining, formation of intratubular multinucleate giant cells, spermatogenic arrest at the spermatocyte stage and decreasing thickness of germinal epithelium layer, thickened basement membranes with interstitial fibrosis and increased peritubular connective tissues were observed in the testicular histopathologic sections of outdoor animals, while there was no histopathologic abnormalities testes of animals kept indoors (Fig. 2).

Serum cortisol concentrations was higher (P<0.05) in outdoor (1.2 ± 0.09 µg/dl) than indoor (0.85 ± 0.09 µg/dl) animals. While there was a slight variation in the monthly serum cortisol concentrations in indoor animals, the outdoor animals showed a monthly significant increase in serum cortisol concentration especially from June toward the end of the experiment (Table 1; P<0.05).

4. Discussion

The results of this study clearly showed an impact of direct exposure of developing lambs to summer heat stress on testicular weight and its microscopic structure without significant effect on serum testosterone concentrations.
The results of the present study on testosterone profile are in agreement with the other studies which reported the low levels of serum or plasma testosterone during developing of ram lambs (Crim and Geschwind, 1972a,b; Varney and Sanford, 1989). The testicular growth of spring born ram lamb increased dramatically from 90 to 150 days of age with a small increase in blood testosterone concentration (1 ng/ml). The serum testosterone concentrations increased considerably (8 ng/ml) from 150 to 200 days of age (Varney and Sanford, 1989). Mean plasma concentration of intact developing ram lambs at 4 month of age was 0.79 ± 0.26 ng/ml (Brown et al., 1988). Schanbacher et al. (1974) showed serum testosterone levels of 0.1–2.0 ng/ml in developing ram lambs, while increased during the puberal period. Lafortune et al. (1984) reported the breeding difference on earlier secretion of LH and production of testosterone in developing ram lambs.

Lower testicular weight and severe damage to testicular microstructures due to chronic heat exposure in the present study are similar to the other reports of heat stress damages on adult males testis (Gomes et al., 1971; Wettmann and Desjardins, 1979; Barenton et al., 1982; Lunstra and Schanbacher, 1988; Sailer et al., 1997). Elevated ambient temperature for 90 days significantly reduced the number of young spermatids in the yearling boar seminiferous tubules without any effect on the number of type A spermatagonia or spermatocytes (Wettmann and Desjardins, 1979). High body temperature in cryptorchid cases induced degeneration of seminiferous tubules in adult ram (Lunstra and Schanbacher, 1988) and completely disrupted the germ cells production (Barenton et al., 1982). On the other hand, exposure of developing ram to summer heat stress did not increase the suppressive effects of photoperiod or their sexual immaturity condition on the serum testosterone profile and confirms the previous reports of serum testosterone levels in bilateral cryptorchid adult ram (Lunstra and Schanbacher, 1988) or long time exposed yearling boar to high ambient temperature (Wettmann and Desjardins, 1979). However, some studies reported the impact of heat stress on testosterone secretion of male animals (Gomes et al., 1971; Sidibe et al., 1992). The differences between the results of the present study with other researches on testosterone profile after heat exposure may be related to the type of heat exposure; chronic or acute.

Can chronic heat exposure in the present study with above effects and pathologies affect the puberty of the developing lamb rams? The future fertility of these kinds of heat exposed animals is out of the scope of the present study. Varney and Sanford (1993) reported that even though testicular diameter or testosterone measurement near the time of lamb ram puberty onset provided the best long-range prediction of adult reproductive function, multi-trait models, e.g., total sperm per ejaculate and ejaculation frequency, are more reliable for a specific age.

Cortisol levels differ between two groups of this experiment with higher levels in outdoor than indoor animals. This finding is in agreement with the previous report of Megahed et al. (2008) who reported the higher levels of serum cortisol during summer than winter months in the female river buffalo. However, higher levels of cortisol after acute heat stress (McMorris et al., 2006) or lower serum cortisol levels during chronic heat stress (Sidibe et al., 1992) have been reported previously. Cortisol levels had a decreasing trend, changes being significant at 10 and 15 weeks after bull scrotal insulation (Sidibe et al., 1992). In contrast to the previous study (Juniewicz et al., 1987) which showed the inhibitory effects of adrenal steroids on ram testicular endocrine function, present study did not show any correlation (r = 0.13; P = 0.18) between cortisol and serum testosterone concentration which may be related to the fact that the experiment was undertook during non-breeding season that the hypothalamus–hypophysis–ovary axis is inactive. The finding of this study is in agreement with that of Stellflug (2006) who found no correlation between testosterone and cortisol concentrations in sexually inactive and sexually active female- and male-oriented rams.

In summary, direct exposure of developing ram lamb to high ambient temperature increased cortisol secretion, with no significant effect on testosterone production and caused severe testicular damage during non-breeding season.

References


