# EFFECT OF ADAPTATION AND HEAT STRESS ON REPRODUCTIVE PERFORMANCES OF FAT-TAIL AWASSI RAMS IN EASTERN MEDITERRANEAN

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***ABSTRACT***

*The Awassi fat-tail sheep is desired in the Middle East and Sahara for its hardiness and resistance. In this region, the adaptability of Awassi rams to elevated heat stress is controversial. The objective of this study was to assess the effect of ambient or testicular 43 ºC heat on semen characteristics. 12 Awassi rams of 1.2 to 2 years were allowed an initial adaptation period (P0), followed by two experimental phases (P1 and P2), each including a period of heat stress (HS1 and HS2) followed by a period of recovery from heat stress (R1 and R2). Heat stress conditions were considered when ambient temperature (Group 2) or testicular environment (Group 3) reached 42 to 44 ºC. Control animals (Group 1) were exposed to ambient environmental conditions. Temperature-humidity index (THI) as a relationship of ambient temperature, wet and dry humidity effect along with respiration and pulse rate, body temperature and live body weight (BW) were measured weekly as adaptation parameters. Reproductive parameters assessed included scrotum circumference and testicular volume measured twice per heat stress period, whereas semen volume, density, concentration and motility were measured twice weekly. Data were analyzed using the mixed procedure of SAS under a time series analysis model, with treatment and time nested within period as main effects, and treatment by time within period as interaction terms. Treatment means were separated using Fisher protected LSD. Results showed a THI of 25.7 during the rest periods (R1 and R2), 30 during HS1 and 28 during HS2. BW decreased with ambient stress, whereas respiration rate increased during heat stress periods (HS1 and HS2). Testes volume decreased with both ambient and testicular heat stress, whereas testicular circumference was not affected by these conditions. Semen concentration decreased whereas semen volume increased with ambient but not testicular heat stress. Spermatozoa abnormalities increased in R1 following heat stress (HS1) mainly in the acrosomal and mid piece regions, but not the tail. Progressive motility was decreased immediately during heat stress periods, but recovered in the rest period. Awassi rams show a potential for heat stress adaptability where the effect of a 1st period of heat stress observed by a decrease in semen characteristics is thereafter dissipated during subsequent heat stress events.*

**Keywords:** heat stress, spermatogenesis, Awassi, fat-tail sheep

## INTRODUCTION

The Awassi breed of sheep in the regions of Lebanon and the desert areas of the Middle East and Arabic peninsula are reared in a vertical and horizontal transhumance. This breed is desired for its milk and meat, but has low quality wool. The Awassi breed is believed to be resistant to heat stress conditions in these regions. Reports on the effect of heat stress conditions on the quality of semen are conflicting where a decrease (Hafez, 1968; Abi Saab & Sleiman, 1986), or no effects (Degen & Shkolink, 1978) of increased temperature on semen quality are reported. Scrotal heat stress caused subfertility and sperm DNA damage in humans (Paul *et al.*, 2008a; Paul *et al.*, 2008b; Perez-Crespo *et al.*, 2008; Paul *et al.*, 2009), mice (Yin *et al.*, 1997; Yaeram *et al.*, 2006), rats (Potemina, 2008), young lambs (Rasooli *et al.*, 2010) and pigs (Wettemann & Bazer, 1985). Previously, techniques for increasing scrotal temperature in rams have been used to study spermatogenesis. These techniques include insulation of the scrotum (Glover, 1955; 1956; Glover & Young, 1963; Byers & Glover, 1984), immersion of scrotum in a water bath with the desired temperature for a short period of time while the animals are under anesthesia or sedation (Mieusset *et al.*, 1991; Setchell *et al.*, 1991; Mieusset *et al.*, 1992; Setchell, 1998) or immobilization (Williamson, 1974). On the other hand, when the heat is localized to the testes, similar semen characteristics and fertility were observed in rams strains with differences in sensitivity to whole body heating (Fowler, 1968). Thus, the objectives were to test the effect of high testicular and ambient temperature on adaptation and semen quality of locally reared Awassi rams using immobilized rams with their testicles over a hot air chamber.

## MATERIALS AND METHODS

### Animals and experimental design

Twelve Awassi rams purchased from multiple regions of Lebanon aged about 1.2 to 2 years were fed free choice hay with a 15 % protein concentrate for the duration of the experiment which lasted from June to October. Three phases for the experiment were determined based on the duration of heat stress as shown in Table 1. Experimental rams were allowed an initial adaptation period (P0), followed by two experimental phases (P1 and P2) to observe the effect of multiple patterns of heat stress on rams' spermatogenic cycle. Each phase included a period of heat stress (HS1 and HS2) followed by a period of recovery from heat stress (R1 and R2), respectively as demonstrated in Table 1.

**TABLE 1**

**Experimental Periods, Durations and Description**

| Period | Treatment | Duration | |
|---|---|---|---|
| P0 | Adaptation period | 28 d | 5/14 – 6/11 |
| HS1 | 6 h heat stress per day | 49 d | 6/12 – 7/25 |
| R1 | Recovery after HS1 | 30 d | 7/26 – 8/26 |
| HS2 | 12 h heat stress per day | 21 d | 8/27 – 9/16 |
| R2 | Recovery after HS2 | 21 d | 9/17 – 10/9 |

Heat stress conditions were considered when ambient temperature (Group 2) or testicular environment (Group 3) reached 42 to 44 ºC, whereas control animals (Group 1) were kept in the same facility under environmental conditions without heat stress or movement restriction. In order to raise testicular environment to the desired temperature, rams were immobilized in chutes with their scrotum placed over heated air as shown in Figure 1.


**Figure 1. Testicular heat stress application to immobilized rams.**
**Panel A: immobilized rams in boxes with source of heat.**
**Panel B: testicular positioning above the heated air source.**

**Measurement of adaptation parameters**

Ambient temperature was measured twice weekly using a dry and wet bulb thermometer, relative humidity was determined following the psychrometer principle from the wet and dry temperatures using the humidity calculator from the Australian bureau of meteorology (http://www.bom.gov.au/lam/humiditycalc.shtml). The temperature-humidy index (THI), reflecting the effective temperature, is thus a tool to measure the severity of heat stress in a region with elevated humidity (Kelly & Bond, 1971; LPHSI, 1990; Finocchiaro *et al.*, 2005; Marai *et al.*, 2007). It was computed using the formula from Finocchiaro *et al.* (2005) for dairy sheep in a Mediterranean region with modification, THI = dbT $^{\circ}$C − {(0.55 − 0.55 RH)x(dbT $^{\circ}$C − 14.4)}, where dbT is the average dry bulb temperature in $^{\circ}$C during the experimental period, and RH is the relative humidity in percentage in that same period. Respiration rate and pulse rate were determined daily *via* counting the pulse beats and breaths respectively, using a stethoscope as previously described (Abi Saab & Sleiman, 1995). Measures were recorded before, during and after stress, once daily during HS1 and twice during HS2 periods. Body temperature was determined daily using a thermometer, whereas body weight was determined bi-weekly using a scale.

**Measurement of reproductive performance parameters**

Scrotum circumference was recorded twice per period *via* measuring the widest point of the testes with a measuring tape. Testicular volume was recorded twice per period as previously described (Oldham *et al.*, 1978). Semen quality of ejaculate was assessed twice per

week; following ejaculate collection *via* electro-ejaculation (4 Hz pulse tension, 3-15 volts for 4 sec), semen density was determined as previously described (Ozin, 1956), and volume was measured in a graduated cylinder. Semen concentration, motility, and abnormality were all assessed on a 400 X magnification under light microscopy as described below. Semen concentration was determined in a hematocytometer in 3 % saline solution, following which semen was extended in locally prepared extender containing 0.8 g/L glucose, 2.8 g/L sodium citrate and 20 % egg yolk in water. Wave motion on a scale of 0 to 10 and progressive motility on a percent basis (Perry, 1968) were then determined with the lowest score corresponding to the least mobile or viable sperm. Semen abnormalitities were determined from a diluted smear stained with a solution of eosin, nigrosin, and sodium citrate in water (Perry, 1968). Abnormal spermatozoa was determined by counting the number that presented abnormalities in their head, mid piece section or tail in a total of 200 spermatozoa.

**Data analysis**

Data for adaptation parameters were analyzed using the mixed procedure of Abi Saab (1998) under a time series analysis model, with treatment and time nested within period as main effects, and treatment by time within period as interaction. Treatment differences were tested using Fisher protected least-significant difference procedure only when interactions were not significant ($P > 0.05$). When interactions were significant, the main effects were separated using the slice option in the least squares means statement. Data were presented as least squares means ± SEM.

Data for semen characteristics and concentration, body weight and body temperature were analyzed using the mixed procedure of Abi Saab (1998), with treatment and period as main effects and treatment by period as interaction. Treatment differences were tested using Fisher protected least-significant difference procedure only when the interaction term was not significant ($P > 0.05$). When interactions were significant, the main effects were separated using the slice option in the LSM statement. Data were presented as least squares means ± SEM.

## RESULTS

**Ambient temperature and humidity**

The temperature humidity index during the rest periods (R1, THI = 25.6 and R2, THI = 22.8) was lower than during the heat stress periods (HS1, THI = 30.0 and HS2, THI = 28.2), clearly delimiting the experimental periods as shown in Figure 2. During the adaptation period (P0), which coïncided with higher temperatures in mid June and early July, the THI was high (29.0).

**Effect of heat stress on adaptation parameters of Awassi rams**

There was no significant effect of treatment by period interaction on rams' body weight; however, body weight was significantly ($P < 0.05$) lower in rams of the heated testicles *vs*. heated room groups as shown in Figure 3A. On the other hand, body weight was significantly ($P < 0.01$) greater at the end of the experiment (R2) than at the beginning periods (adaptation, HS1 and R1) as shown in Figure 3B.

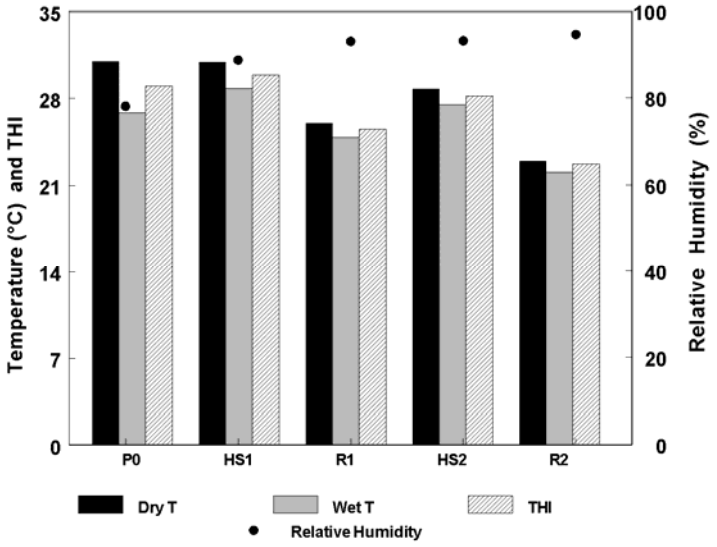


**Figure 2. Monthly averages of meteorological data during the experimental period from June to October. Dry bulb temperature (Dry T, °C), wet bulb temperature (Wet T, °C), temperature-humidity index (THI), average relative humidity (RH, %).**

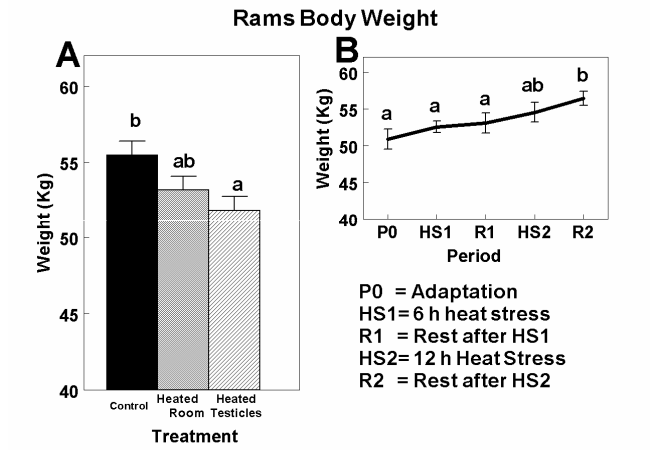


**Figure 3. Average body weight of rams. [a, b] Within each panel, means with different subscripts differ (P < 0.05).**

The Awassi rams were able to dissipate the increase in environmental temperature in HS1 without allowing it to affect their body temperature. However, following a morning heat stress (HS2 - am), the rams were not able to deal with heat stress and their body temperature increased significantly ($P < 0.05$) following the afternoon 6 h heat stress (HS2 - pm) as shown in Figure 4. Rams' body temperature stayed significantly ($P < 0.05$) higher in the last rest period (R2) than R1 or HS2 - am or even HS1 as shown in Figure 4.

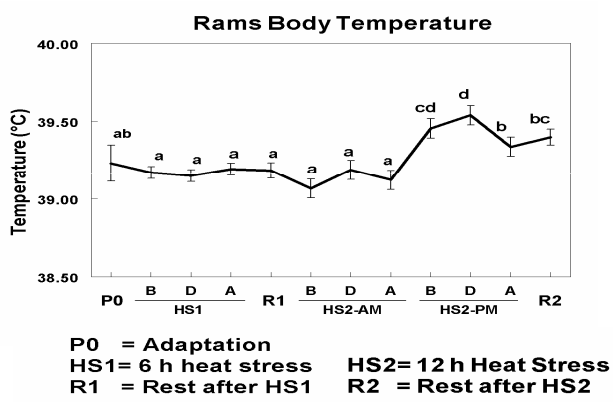


**Figure 4. Body temperature across treatments of rams exposed or not to the different heat stress conditions and periods. [a, b, c] Means with different subscripts differ ($P < 0.05$).**

There was a significant ($P < 0.05$) effect of treatment by time within period interaction on respiration rate in Awassi rams. When this interaction was sliced by treatment, response differed significantly ($P < 0.05$) within the control and heated room groups, but not within the heated testicles group as shown in Figure 5. In fact, the control rams only showed a significant ($P < 0.05$) increase in respiration rate at the beginning of the 2nd period of morning heat stress (HS2) as compared to the respiration rate in all other periods and times as shown in Figure 5. Within the heated room group, the respiration rate increased significantly ($P < 0.05$) whenever heat stress was applied (HS1, HS2 morning and evening). This respiration rate returned to normal thereafter and stayed as such during the rest periods (R1 and R2). Pulse rate did not differ significantly ($P > 0.05$) between treatment groups or periods (Figure 6). Even though differences were expected to mimic respiration rate differences, however, the large variability within the data did not allow the significant separation of the means.

**Effect of heat stress on rams testes**

There was no significant ($P > 0.05$) effect of treatment by period interaction on either the average of testes circumference (Figure 7) or volume (Figure 8). Testes circumference did not change ($P > 0.05$) between test groups (Figure 7A), but was significantly decreased ($P < 0.05$) during HS1 as shown in Figure 7B. However, testes volume significantly decreased ($P < 0.05$) in both heat stressed groups as shown in Figure 8A, whereas HS1 caused a decrease ($P < 0.05$) that was seen during R1; the testes volume recovered thereafter as shown in Figure 8B.

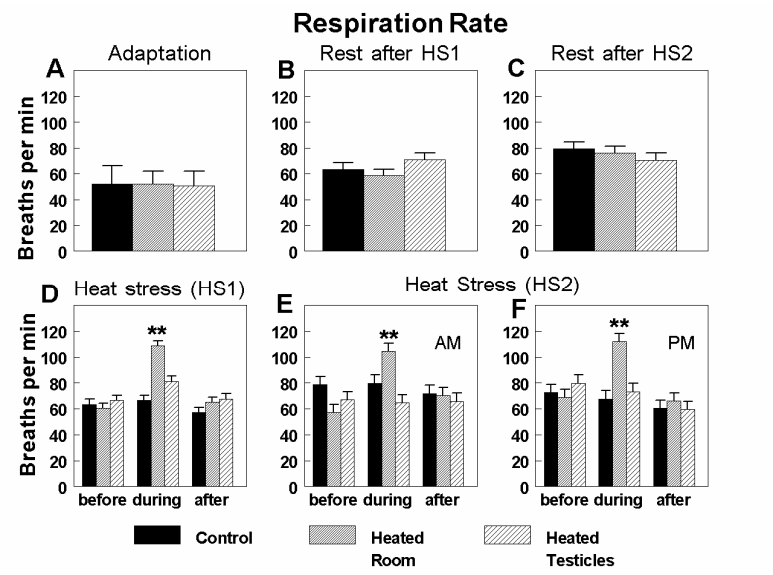


**Figure 5. Respiration rate in rams deprived of heat stress (control), environmental heat stress (heated room), or testicular heat stress (heated testicles), during adaptation (Panel A), 1st period of 6h of heat stress (HS1 – Panel D), rest after HS1 (Panel B), 2nd period of heat stress (HS2) applied 6h in the morning (AM – Panel E) and 6 h in the afternoon (PM – Panel F), and rest after HS2 (Panel C).**
**** LSM significantly different from all the other means (P < 0.05).**

**Effect of heat stress on semen quality and evaluation**

There was no significant (P > 0.05) treatment by period interaction on either semen volume (Figure 9), or semen concentration (Figure 10). Semen volume was not affected (P > 0.05) by the heat stress treatment method as shown in Figure 9A. However, semen volume was significantly (P < 0.05) increased in R1 and HS2 above HS1 and restored to lowest levels in R2 as shown in Figure 9B. On the other hand, semen concentration was decreased (P = 0.07) in heated room group *vs*. both the control and heated testicle groups as shown in Figure 10A. Semen concentration was significantly decreased (P < 0.05) following the 1st period of heat stress (HS1) as shown in R1, Figure 10B. Semen concentration was not restored during the first period of rest (R1) and was further decreased during the 2nd heat stress period (HS2), and stayed as such until the end of the experiment as shown in Figure 10B.

The percent of normal spermatozoa (Figure 11A) decreased (P < 0.05) in R1, as a result of the heat stress period HS1. This number recovered (P < 0.05) and reached normal levels during HS2 and was not further affected (P > 0.05) by heat stress. The spermatozoa

abnormalities increased ($P < 0.05$) mainly in the acrosome (Figure 11B) and mid-piece (Figure 11C) and were seen starting HS1 and continued to the rest period R1. The number of spermatozoa abnormalies decreased ($P < 0.05$) thereafter and was not further affected ($P > 0.05$) by the subsequent heat stress period as shown in Figure 11B and C. This observation of the increased number of abnormalities during HS1 might be due to the increased THI index during P0, which affected the presence of abnormalities rather than the total number of normal spermatozoa as shown in Figure 11. No significant differences ($P > 0.05$) in tail abnormalities were observed as a result of heat stress as shown in Figure 11D.

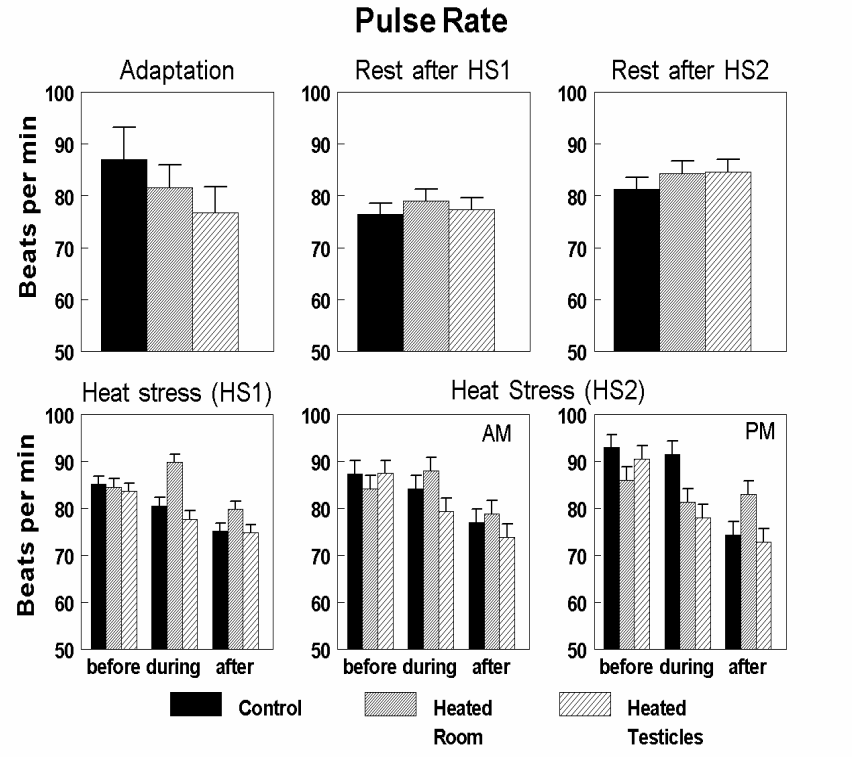


**Figure 6. Pulse rate of rams deprived of heat stress (control), environmental heat stress (heated room), or testicular heat stress (heated testicles) during adaptation (Panel A), 1st period of 6h of heat stress (HS1 – Panel D), rest after HS1 (Panel B), 2nd period of heat stress (HS2) applied 6h in the morning (AM – Panel E) and 6 h in the afternoon (PM – Panel F), and rest after HS2 (Panel C). No significant difference was observed among all means ($P > 0.10$).**

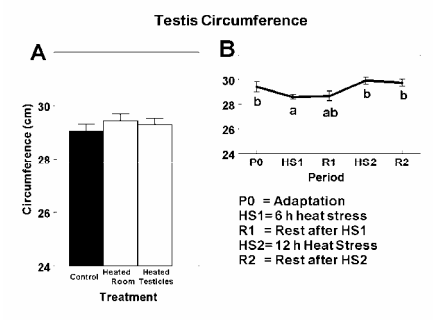


**Figure 7. Effect of heat stress on average testes circumference of rams. [a, b] Within each panel, means with different subscripts differ (P < 0.05).**

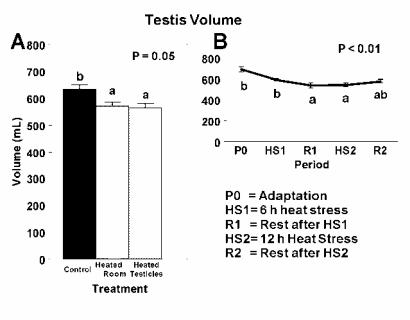


**Figure 8. Effect of heat stress on average testes volume of rams. [a, b] Within each panel, means with different subscripts differ (P ≤ 0.05).**

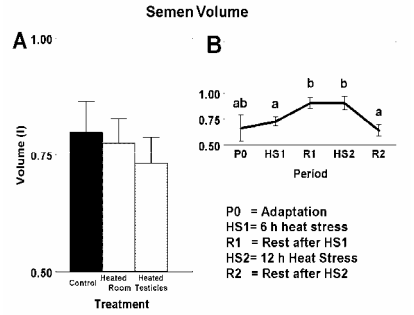


**Figure 9. Effect of heat stress on average ejaculate volume in rams. [a, b] Within each panel, means with different subscripts differ (P < 0.05).**

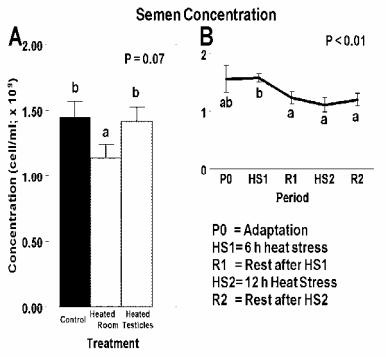


**Figure 10. Effect of heat stress on average ejaculate semen concentration in rams. [a, b] Within each panel, means with different subscripts differ.**

There was no significant effect of heat stress periods ($P > 0.05$), treatment ($P > 0.05$) or their interaction ($P > 0.05$) on average mass motility of ram semen (data not shown). Also, there was no significant ($P > 0.05$) treatment by period interaction on average semen motility. Progressive motility was not affected ($P > 0.05$) by treatment as shown in Figure 12A, but the decrease in progressive motility during the 1st heat stress period (HS1) was significantly ($P < 0.05$) restored during the rest period (R1) as shown in Figure 12B. Rams seem to have adapted to the heat stress since there was no further significant effect of the 2nd heat stress period on the progressive motility of semen in HS2 as shown in Figure 12B.

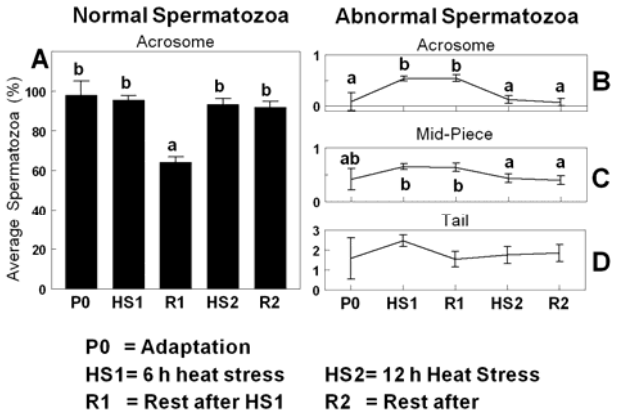


**Figure 11. Effect of heat stress on average percent of normal and abnormal spermatozoa.**
**[a, b] Means without a common subscript differ (P < 0.01).**

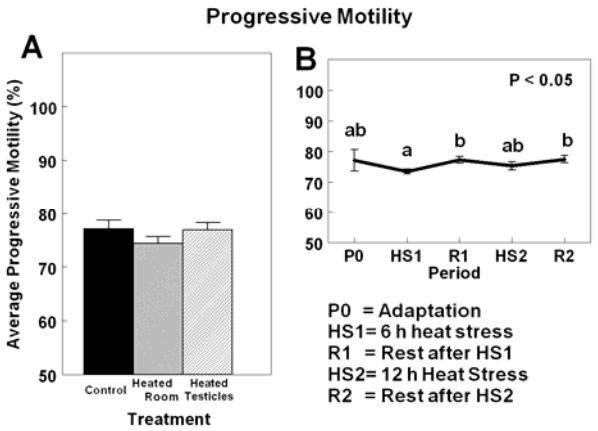


**Figure 12. Effect of heat stress on average progressive semen motility in rams.**
**[a, b] Within each panel, means with different subscripts differ (P < 0.05).**

## DISCUSSION

Awassi rams are adapted to local conditions in Lebanon and the Eastern Mediterranean that include periods of summer heat stress with elevated relative humidity. In fact, this experiment shows that housing the rams indoors during the hot months of August

and September relieves rams and decreases the effective ambient temperature, regardless of the high relative humidity. Shearing (Dutt & Hamm, 1957; Eyal, 1963c; Austin & Young, 1977), in addition to providing shade or shelter (Hofman & Riegle, 1977; Marai *et al.*, 2001; Finocchiaro *et al.*, 2005; Marai *et al.*, 2007) during summer has been suggested to relieve heat stress. In this study, rams body weight as a measure of performance was not affected by heat stress, possibly due to the sheltering and the low temperature humidity index during the hottest months of summer. In humid Mediterranean climate where the effective temperature tends to increase with increased solar exposure, the dissipation of heat by increased respiration rate can be effective (Finocchiaro *et al.*, 2005). However, in this experiment, localized testicle heating decreased rams body weight, possibly indicating a different mechanism to localized heat dissipation as compared to whole body adaptation to heat stress. Some authors (Meitner, 1976; Kholkute & Udupa, 1979; McGrady, 1984; Almeida *et al.*, 1998; Almeida *et al.*, 2000; Rai *et al.*, 2003; Potemina, 2008) suggested a decreasing effect of immobilization on spermatogenesis and body weight. In this study, immobilization of rams for 6 h (HS1) or 12 h (HS2) per day over the heat chambers, in addition to the heat stress caused a decrease in body weight. However, this data supports the hypothesis of a different heat stress dissipation mechanisms evidenced by increased respiration and pulse rates during both heat stress periods in the whole-body stressed animals (heated room - Group 1) as compared to the testicle-localized heat stressed animals (heated testicles - Group 2). In fact, in Awassi sheep, the respiration (Eyal, 1963b) and pulse (Eyal, 1963a) rates were at their highest during the hottest periods of the day.

In the current study, one did not observe a change in testicular circumference as a result of heat stress, although a significant decrease of testicular volume in heat stressed animals was observed. At the same time, an increase in ejaculated semen volume as a result of heat stress was observed. Together, these results suggest the possibility of the loss of water by the testes as a result of heat stress, an event causing a change in semen quality. Previously, 45 min of heat stress at 42 ºC over a period of 20 d caused a decrease in testicular volume (Setchell *et al.*, 1991; Hochereau-de Reviers *et al.*, 1993; Setchell, 1998), testicular weight (Setchell *et al.*, 1991; Hochereau-de Reviers *et al.*, 1993) and number of all germ cells except A0 spermatogonia (Hochereau-de Reviers *et al.*, 1993).

In addition, this study showed a decrease in the spermatozoa concentration as a result of a heated environment rather than heated testes, suggesting that stressful environment affects spermatogenesis at a much greater extent than when testes are exposed to localized heat, indicating a major role of the endocrine system and animal thermoregulation. The decrease in the number of spermatozoa was reported to occur in a short period (10 to 12 days) following heat stress (Dutt & Bush, 1955; Waites, 1961; Setchell *et al.*, 1991; Smith & Merilan, 1991), levels returning to normal only a month or so later (Marai *et al.*, 2007). In mice, spermatogenesis was severely affected by higher temperatures and heat stress (Cataldo *et al.*, 1997). However, the number of abnormalities was greater following localized testes heating than in whole body heat stress, thus indicating lower quality semen from these rams and an overall decrease in fertility.

The effect of heat stress observed following HS1 was not seen following HS2, possibly due to the adaptation of the animal to heat stress. In fact, scrotal adaptation to heat stress involves the development of sweat glands, mainly following a prior exposure to heat stress (Setchell, 1998), thus allowing for a better dissipation of the effects of heat stress on spermatogenesis.

## CONCLUSION

The study showed an increase in adaptation parameters, mainly respiration rate and pulse rate, as a response to heat stress, an overall decrease in fertility of rams exposed to heat stress and a delayed effect of heat stress, where heat stress effects are observed after a certain period of time. On the other hand, it was also observed that the Awassi ram has a great ability to heat stress adaptation where an exposure to heat stress allows the development of adaptation mechanisms and causes no further effect of subsequent heat stress on spermatogenesis.

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