

## Effects of Exhaustion Test on Semen Characteristics and Blood Composition of Shaded and Unshaded Sudanese Desert Rams (*Ovis aries*)

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**Abstract:** This study was designed to investigate the effects of exhaustion test (frequent ejaculation) on blood composition and semen characteristics of shaded and unshaded Hamari desert rams. Eight rams were divided into two groups, shaded (n=4) and unshaded (n=4) which were exposed to direct solar radiation for five weeks. The exhaustion test was performed for both group alternatively every 30 min. in the 4<sup>th</sup> and 5<sup>th</sup> week of the experiment for three successive days. Blood samples were collected pre and post-exhaustion test and analyzed as well as semen samples. In both groups, exhaustion test significantly lowered the total leukocytes count (TLC), packed cell volume (PCV), total serum protein (STP) and plasma glucose (PLG) levels. However, in shaded rams the monocytes ratio and serum urea (SU) concentration were significantly increased and the eosinophils ratio significantly decreased. Frequent ejaculation significantly lowered, the ejaculate volume (EV), sperm individual motility (SIM) sperm cell concentration (SCC) and increased abnormal sperm (ABS) percent and semen pH of shaded rams. Exhaustion test of unshaded rams significantly increased the SIM in the 1<sup>st</sup> day, life sperm percent (LSP) and SCC in the 3<sup>rd</sup> day and ABS in the 2<sup>nd</sup> day. However, it significantly decreased EV, SCC in the 1<sup>st</sup> day, ABS in the 1<sup>st</sup> and 3<sup>rd</sup> day and SIM in the last day. It is concluded that the frequency of ejaculation had impacts on semen characteristics and blood parameters of shaded and unshaded desert rams.

**Key words:** Exhaustion Test Blood Semen Characteristics Desert Rams

### INTRODUCTION

In the tropics, under the nomadic system of sheep rearing, heat stress affects the physiological responses [1] and productive abilities [2] of rams. Under this extensive system, the reproductive performance of rams is influenced by thermal load [3, 4] as they have to combat heat and serve females throughout the year. Rams can serve several times a day when newly introduced to ewes; they maintain a higher frequency of mating (2-3 times in few minutes), but they tend to mate at a rate of once every 1-5 hours when continuously kept with ewes on heat [5]. Frequent ejaculation affects the sexual activity and semen characteristics of rams [6, 7]. However, there are no problems in maintaining a continuous sexual drive due to their small ejaculate and large epididymal reserve [7], which is important in rams. Frequent ejaculation indicates the superior mating ability of the male, which is important in rams when female-to-male ratio is relatively high [8].

In rams, more frequent ejaculation, probably to the point of exhaustion and refusal of service is necessary for the assessment of ejaculation capacity and semen characteristics [9]. Previous studies indicated that semen characteristics [1] and blood parameters [10] of Hamari desert rams are influenced by exposure to direct solar heat. This work was executed to assess the degree to which shaded and heat stressed desert Hamari rams may be used in intensive breeding programs and its effects on semen characteristics and some blood parameters.

### MATERIALS AND METHODS

**Climate and Location:** This study was done in the Faculty of Veterinary Medicine, University of Khartoum, Shambat, Located at 15°36' N, 32°35' E and an altitude of 390 m. The climatic data prevailing during the experimental period were obtained from Shambat Meteorological Centre located about 500 meters from the experimental site.

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**Animals:** Eight adult intact Desert rams aged 2-3 years were randomly divided into two groups of 4 rams each. Group A was kept under shade (shaded) and group B was exposed to direct solar radiation (Unshaded). The unshaded group was represented by 3 rams due to the sudden death of one ram before semen collection.

**Experimental Procedure:** Both groups of rams had an adaptation period of 2 weeks during which they were exposed to new rearing and housing programs followed by an experimental period of 5 weeks. The experimental period included deworming, clinical examination, claws trimming, general health and breeding soundness evaluation of rams. Animals were fed chopped lucerne hay (*Medicago Sativa*) (CP:17.5%; ME:8.48 MJ/Kg) and were given tap water *ad libitum*.

**Collection and Manipulation of Samples:** In the 4<sup>th</sup> and 5<sup>th</sup> week of the experimental period, the exhaustion test was performed for both groups of rams alternatively. Semen samples were collected every 30 min. from each ram in three consecutive days using an electro-ejaculator ((The Ruakura MK1 V Ram ejaculator; Alfred Cox, Surrey, England) according to the method adopted [11, 12]. From each ram, during the exhaustion test, blood samples were drawn before the collection of the first semen sample (pre-exhaustion) and after the last one (post-exhaustion) under aseptic conditions from the jugular vein using plastic disposable syringes. A sample of total 5 ml was manipulated following the same steps adopted in blood analysis in desert Hamari rams [10] for the determination of total (TLC) and differential (DLC) leukocytes count, packed cell volume (PCV) and serum concentration of total proteins (STP), albumins (SAB), urea (SU) and plasma glucose (PLG).

#### Analysis of Samples

**Blood Samples:** The analysis of blood samples were carried out according to the standard methods described in Schalm's Veterinary Haematology [13]. The serum concentrations of total protein (STP), albumin (SA) and urea (SU) were determined according to the protocol applied in desert Hamari rams [10].

**Semen Samples:** Semen samples were analyzed according to the standard method adopted by Boundy [12] for the determination of ejaculate volume (EV), sperm mass (SMM) and individual (SIM), motility, sperm concentration (SCC), live (LSP) and abnormal sperms (ABS) percent and semen pH and similar to the method carried out in semen evaluation of Hamari desert rams [1].

**Statistical Analyses:** The effects of exhaustion test on semen characteristics and blood composition were evaluated by two way ANOVA using the statistical software [14]. The data obtained were presented as means  $\pm$  S.E.

## RESULTS AND DISCUSSION

The prevailing climatic data are depicted in Table 1.

Table 4 shows that the post-exhaustion (POE) values of packed cell volume (PCV) level for the shaded rams, were significantly ( $p < 0.05$ ) lower during the three days of the experiment. This could be attributed to alterations in vasopressin secretion and water balance in response to cortisol secretion. Parker *et al.* [15] indicated that in stressed sheep, plasma cortisol increases and enhances the secretion of vasopressin and a consequent reduction in water loss occurs, resulting in low PCV. The decrease in PCV could be also accounted for by a transient expansion of plasma volume to defend against water loss during frequent ejaculation and heat stress through sweating and panting. Erickson [16] reported that an increase of plasma volume in equines and athletes in response to endurance exercise may serve to defend against excessive water loss during protracted work and heat stress by induced hypervolaemia as a negative water balance from increased water loss. However, the significant ( $p < 0.05$ ) reduction in PCV level observed in the POE state during the 3<sup>rd</sup> day in the unshaded rams (Table 5), is clearly related to haemodilution which resulted from heat stress, increased water consumption and accentuation by excess muscle contraction [17] associated with frequent ejaculation. Similar reduction in TLC level, which did not attain significant level, was reported [10].

Exhaustion test significantly ( $P < 0.05$ ) lowered the total leukocyte count (TLC) of the shaded rams, in the 1<sup>st</sup> and 2<sup>nd</sup> day (Table 4). This response could be attributed to the effects of increase in the secretion of adrenocortical steroids. Swenson [18] indicated that adrenocorticoids may cause an increase in antibodies concentration in the blood through dissolution of lymphocytes into lymphoid tissue resulting in lymphopenia. The reduction in TLC could also be related to the increase in blood and lymph flow to the contracting muscles. It was indicated that during exercise, the increase in blood flow to the active muscles was associated with vasodilatation [19]. In unshaded rams, the significant reduction in TLC in the 1<sup>st</sup> and 3<sup>rd</sup> day of the experimental period (Table 5) could be related to the combined effects of prolonged heat stress and frequent ejaculation. Similar reduction in PCV was reported in unshaded Hamari rams [10].

Table 1: The prevailing climatic conditions during the experimental period

| Time (weeks) | Temperature (°C) |      |       | RH (%) (Mean) |
|--------------|------------------|------|-------|---------------|
|              | Max.             | Min. | Mean  |               |
| 1            | 35.6             | 19.7 | 27.7  | 23            |
| 2            | 38.5             | 20.6 | 29.6  | 25            |
| 3            | 36.5             | 20.2 | 28.4  | 26            |
| 4            | 32.9             | 18.0 | 25.5  | 39            |
| 5            | 34.2             | 15.9 | 25.1  | 30            |
| Mean         | 35.15            | 18.9 | 27.26 | 28.6          |
| ±SE          | ±1.40            | ±1.7 | ±1.72 | ±5.68         |

Solar radiation: 377 W/m<sup>2</sup>

Table 2: Effect of exhaustion test on differential leukocyte count, DLC (%) in shaded Desert rams. (n = 24)

| Cell type (%) | Time (Days) | PRE                       | POE                       |
|---------------|-------------|---------------------------|---------------------------|
| Lymphocytes   | 1           | 58.75 <sup>Aa</sup> ±1.65 | 62.50 <sup>Aa</sup> ±1.04 |
|               | 2           | 58.25 <sup>Aa</sup> ±2.59 | 57.25 <sup>Aa</sup> ±1.97 |
|               | 3           | 53.00 <sup>Aa</sup> ±5.61 | 55.50 <sup>Aa</sup> ±2.40 |
| Neutrophils   | 1           | 31.25 <sup>Aa</sup> ±1.31 | 30.50 <sup>Aa</sup> ±1.32 |
|               | 2           | 34.00 <sup>Aa</sup> ±1.96 | 31.25 <sup>Aa</sup> ±1.25 |
|               | 3           | 37.00 <sup>Aa</sup> ±6.22 | 32.00 <sup>Aa</sup> ±2.38 |
| Eosinophils   | 1           | 4.22 <sup>Aa</sup> ±2.02  | 2.25 <sup>Aa</sup> ±0.25  |
|               | 2           | 3.75 <sup>Aa</sup> ±1.03  | 3.00 <sup>Aa</sup> ±0.91  |
|               | 3           | 4.25 <sup>Aa</sup> ±1.11  | 3.00 <sup>Ab</sup> ±0.71  |
| Monocytes     | 1           | 5.75 <sup>Aa</sup> ±0.75  | 4.76 <sup>Ba</sup> ±0.25  |
|               | 2           | 4.11 <sup>Aa</sup> ±0.41  | 8.50 <sup>Aa</sup> ±2.33  |
|               | 3           | 5.75 <sup>Aa</sup> ±1.11  | 9.25 <sup>Aa</sup> ±2.17  |

Values (mean±SE), for each parameter, with different lower case letters in the same row and with different upper case letters in the same Column differ significantly (P < 0.05)

Table 3: Effect of exhaustion test on differential leukocyte count, DLC (%), in unshaded Desert rams (n = 18)

| Cell type (%) | Time (Days) | PRE                        | POE                        |
|---------------|-------------|----------------------------|----------------------------|
| Lymphocytes   | 1           | 46.67 <sup>Aa</sup> ±3.84  | 50.67 <sup>Aa</sup> ±6.36  |
|               | 2           | 51.67 <sup>Aa</sup> ±7.53  | 51.67 <sup>Aa</sup> ±2.48  |
|               | 3           | 52.67 <sup>Aa</sup> ±8.83  | 48.00 <sup>Aa</sup> ±10.97 |
| Neutrophils   | 1           | 46.67 <sup>Aa</sup> ±4.26  | 43.33 <sup>Aa</sup> ±6.17  |
|               | 2           | 40.67 <sup>Aa</sup> ±8.84  | 38.33 <sup>Aa</sup> ±3.53  |
|               | 3           | 43.33 <sup>Aa</sup> ±10.87 | 47.00 <sup>Aa</sup> ±11.63 |
| Eosinophils   | 1           | 1.67 <sup>Aa</sup> ±0.67   | 1.33 <sup>Aa</sup> ±0.33   |
|               | 2           | 2.33 <sup>Aa</sup> ±0.33   | 3.83 <sup>Aa</sup> ±0.33   |
|               | 3           | 0.67 <sup>Aa</sup> ±0.67   | 3.00 <sup>Aa</sup> ±0.05   |
| Monocytes     | 1           | 3.67 <sup>Aa</sup> ±0.88   | 4.67 <sup>Aa</sup> ±1.76   |
|               | 2           | 5.33 <sup>Aa</sup> ±1.20   | 8.67 <sup>Aa</sup> ±0.88   |
|               | 3           | 3.67 <sup>Aa</sup> ±1.45   | 4.00 <sup>Aa</sup> ±0.01   |

Values (mean±SE), for each parameter, with different lower case letters in the same row and with different upper case letters in the same Column differ significantly (P < 0.05)

For the shaded rams, the significant (P < 0.05) reduction in eosinophils ratio was observed in the 3<sup>rd</sup> day POE (Table 2) could be associated with the high levels of adrenocorticotrophic hormones (ACTH) secreted in response to stress. However, the ratio of monocytes was significantly (P < 0.05) higher in the last two days POE (Table 2). This response could be attributed to the reduction in ACTH level and cortisol secretion as the reduction in monocytes ratio in the first day of exhaustion may indicate the predominance of ACTH. Previous work indicated that, high cortisol level reduced the ratio of eosinophils and monocytes [16]. Frequent ejaculation significantly decreased the serum total protein level of the shaded rams in the 2<sup>nd</sup> and 3<sup>rd</sup> day of the experimental period (Table 4). This could be attributed to increased lymph flow during frequent ejaculation. Ganong [19] indicated that during exercise, the lymph flow is greatly increased, limiting the accumulation of interstitial fluid in an effect greatly increasing its turnover. Also this response could be related to cortisol effects and the breakdown of nucleic acid in muscles [17]. In the unshaded rams, the significant reduction (p < 0.05) in total protein level in the 1<sup>st</sup> and 2<sup>nd</sup> day POE (Table 5) could be associated with an increase in water intake and haemodilution, in addition to stress induced by frequent ejaculation.

The significant (p < 0.05) increase in serum urea concentration reported in the 3<sup>rd</sup> day in the shaded rams POE (Table 4), could be related to the increase in the rate of protein catabolism. Erickson [16] indicated that in exercise in endurance horses the consistent increase in plasma urea level is primarily attributed to increase in the rate of protein catabolism. This finding is in agreement with what was reported in the literature [17].

Successive semen collection significantly (p < 0.05) increased the glucose level of shaded rams (Table 4) in the 1<sup>st</sup> and 3<sup>rd</sup> day. This stressful condition, induces an increase in the level of glucocorticoids which influences the plasma concentrations of energy substrates including glucose. The actions of glucocorticoids include increased hepatic glucogenesis and gluconeogenesis [17]. Glucose 6-phosphate activity is increased and the plasma glucose level increases. Glucocorticoids exert an anti-insulin action in the peripheral tissues [16, 20], which increases the blood glucose level. For the unshaded rams, there was a significant (p < 0.05) increase in plasma glucose level POE during the three days (Table 5). This response could be attributed to the combined effects of heat stress and exhaustion test, as heat stress increases gluconeogenesis [21].

Table 4: Effects of exhaustion test on some blood parameters of shaded Desert rams (n = 24)

| Blood parameters |                        |                        |  |                       |                        |                        |                           |                       |                       |                       |                        |                        |
|------------------|------------------------|------------------------|--|-----------------------|------------------------|------------------------|---------------------------|-----------------------|-----------------------|-----------------------|------------------------|------------------------|
| Time/Days        | PVC%                   |                        | Total leukocyte count TLC( $\times 10^3/\mu\text{l}$ ) |                       | Glucose conc. (mg/dl)  |                        | Total protein con. (g/dl) |                       | Albumin conc. (g/dl)  |                       | Urea conc. (mg/dl)     |                        |
|                  | PRE                    | POE                    | PRE  | POE                   | PRE                    | POE                    | PRE                       | POE                   | PRE                   | POE                   | PRE                    | POE                    |
| 1                | 34.33 <sup>±1.86</sup> | 27.67 <sup>±1.76</sup> | 6.28 <sup>±1.51</sup>                                  | 3.98 <sup>±0.57</sup> | 51.03 <sup>±5.26</sup> | 58.40 <sup>±2.28</sup> | 7.63 <sup>±0.35</sup>     | 7.68 <sup>±0.33</sup> | 3.50 <sup>±0.12</sup> | 3.65 <sup>±0.05</sup> | 17.45 <sup>±2.85</sup> | 17.20 <sup>±3.20</sup> |
| 2                | 35.50 <sup>±1.89</sup> | 31.25 <sup>±2.66</sup> | 8.46 <sup>±0.24</sup>                                  | 4.14 <sup>±0.44</sup> | 54.88 <sup>±0.75</sup> | 58.50 <sup>±4.30</sup> | 7.88 <sup>±0.29</sup>     | 7.35 <sup>±0.32</sup> | 3.65 <sup>±0.12</sup> | 3.65 <sup>±0.16</sup> | 18.38 <sup>±3.17</sup> | 21.75 <sup>±2.29</sup> |
| 3                | 34.33 <sup>±2.03</sup> | 29.33 <sup>±3.33</sup> | 4.50 <sup>±0.26</sup>                                  | 5.36 <sup>±0.23</sup> | 57.05 <sup>±3.02</sup> | 62.23 <sup>±4.51</sup> | 7.20 <sup>±0.29</sup>     | 6.60 <sup>±0.19</sup> | 3.38 <sup>±3.75</sup> | 3.75 <sup>±0.21</sup> | 21.08 <sup>±3.48</sup> | 28.40 <sup>±0.16</sup> |

Values (mean $\pm$ SE), for each parameter, with different lower case letters in the same row and with different upper case letters in the same Column differ significantly ( $P < 0.05$ )

Table 5: Effects of exhaustion test on some blood parameters of Unshaded Desert rams (n = 18)

| Blood parameters |                        |                        |  |                       |                        |                        |                           |                       |                       |                       |                        |                        |
|------------------|------------------------|------------------------|--|-----------------------|------------------------|------------------------|---------------------------|-----------------------|-----------------------|-----------------------|------------------------|------------------------|
| Week             | PVC%                   |                        | Total leukocyte count TLC( $\times 10^3/\mu\text{l}$ ) |                       | Glucose conc. (mg/dl)  |                        | Total protein con. (g/dl) |                       | Albumin conc. (g/dl)  |                       | Urea conc. (mg/dl)     |                        |
|                  | PRE                    | POE                    | PRE  | POE                   | PRE                    | POE                    | PRE                       | POE                   | PRE                   | POE                   | PRE                    | POE                    |
| 1                | 31.25 <sup>±1.75</sup> | 28.25 <sup>±2.46</sup> | 6.88 <sup>±0.98</sup>                                  | 5.75 <sup>±1.35</sup> | 50.93 <sup>±9.45</sup> | 66.43 <sup>±5.86</sup> | 7.73 <sup>±0.43</sup>     | 7.13 <sup>±0.63</sup> | 3.70 <sup>±0.06</sup> | 3.77 <sup>±0.22</sup> | 17.67 <sup>±0.63</sup> | 17.10 <sup>±1.78</sup> |
| 2                | 32.15 <sup>±3.10</sup> | 28.67 <sup>±2.67</sup> | 6.98 <sup>±0.82</sup>                                  | 7.68 <sup>±1.60</sup> | 54.00 <sup>±5.12</sup> | 71.87 <sup>±6.62</sup> | 7.77 <sup>±0.03</sup>     | 6.93 <sup>±0.33</sup> | 3.67 <sup>±0.17</sup> | 3.47 <sup>±0.13</sup> | 16.80 <sup>±4.18</sup> | 14.43 <sup>±1.79</sup> |
| 3                | 30.50 <sup>±1.71</sup> | 26.13 <sup>±0.71</sup> | 5.78 <sup>±0.86</sup>                                  | 4.85 <sup>±1.45</sup> | 50.00 <sup>±3.41</sup> | 67.13 <sup>±7.71</sup> | 7.33 <sup>±0.29</sup>     | 6.77 <sup>±0.24</sup> | 3.40 <sup>±0.12</sup> | 3.63 <sup>±0.18</sup> | 18.93 <sup>±2.58</sup> | 15.57 <sup>±1.85</sup> |

Values (mean $\pm$ SE), for each parameter, with different lower case letters in the same row and with different upper case letters in the same Column differ significantly ( $P < 0.05$ )

Table 6: Effect of exhaustion test on semen characteristics of shaded Desert rams (n = 54)

| Time/Day | Ejaculate volume EV(ml) |                       | Mass motility (MM)    |                       | Individual motility (SIM) |                         | Cell conc. (SSC) ( $\times 10^6/\text{ml}$ ) |                       | live sperm (LSP)%      |                        | Abnormal sperm (ABS)% |                       | Semen pH              |                       |
|----------|-------------------------|-----------------------|-----------------------|-----------------------|---------------------------|-------------------------|--|-----------------------|------------------------|------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|          | 1 <sup>st</sup>         | Last                  | 1 <sup>st</sup>       | Last                  | 1 <sup>st</sup>           | Last                    | 1 <sup>st</sup>                              | Last                  | 1 <sup>st</sup>        | Last                   | 1 <sup>st</sup>       | Last                  | 1 <sup>st</sup>       | Last                  |
| 1        | 1.50 <sup>±0.20</sup>   | 0.50 <sup>±0.10</sup> | 3.25 <sup>±0.25</sup> | 3.50 <sup>±2.50</sup> | 57.50 <sup>±10.31</sup>   | 42.50 <sup>±2.50</sup>  | 2.33 <sup>±0.30</sup>                        | 1.31 <sup>±0.24</sup> | 98.53 <sup>±1.01</sup> | 99.65 <sup>±0.35</sup> | 4.65 <sup>±3.23</sup> | 2.30 <sup>±1.30</sup> | 7.08 <sup>±0.08</sup> | 8.00 <sup>±0.00</sup> |
| 2        | 1.28 <sup>±0.19</sup>   | 0.33 <sup>±0.13</sup> | 3.13 <sup>±0.31</sup> | 2.00 <sup>±0.58</sup> | 37.50 <sup>±4.33</sup>    | 35.00 <sup>±5.00</sup>  | 1.42 <sup>±0.28</sup>                        | 0.61 <sup>±0.70</sup> | 98.88 <sup>±0.54</sup> | 98.63 <sup>±0.81</sup> | 1.35 <sup>±0.16</sup> | 4.97 <sup>±2.95</sup> | 7.88 <sup>±0.13</sup> | 8.00 <sup>±0.01</sup> |
| 3        | 0.87 <sup>±0.09</sup>   | 0.30 <sup>±0.01</sup> | 2.67 <sup>±0.33</sup> | 2.75 <sup>±0.75</sup> | 33.33 <sup>±12.02</sup>   | 16.67 <sup>±11.67</sup> | 1.07 <sup>±0.46</sup>                        | 0.98 <sup>±0.15</sup> | 99.47 <sup>±0.29</sup> | 97.30 <sup>±0.10</sup> | 2.37 <sup>±0.29</sup> | 2.05 <sup>±1.15</sup> | 7.83 <sup>±0.12</sup> | 7.75 <sup>±0.25</sup> |

Values (mean $\pm$ S.E.) within the same row bearing similar lowercase (small) letters and values within the same column bearing similar uppercase (capital) letters are not significantly different at  $p < 0.05$ .

Table 7: Effect of exhaustion test on semen characteristics of Unshaded Desert rams(n = 38)

|   | Ejaculate volume (EV/ml) |                       | Mass motility (MM)    |                       | Individual motility (SIM) |                         | Cell conc. (SSC) ( $\times 10^6/\text{ml}$ ) |                       | Live sperm (LSP)%      |                        | Abnormal sperm (ABS)%   |                        | Semen pH              |                       |
|---|--------------------------|-----------------------|-----------------------|-----------------------|---------------------------|-------------------------|--|-----------------------|------------------------|------------------------|-------------------------|------------------------|-----------------------|-----------------------|
|   | 1 <sup>st</sup>          | Last                  | 1 <sup>st</sup>       | Last                  | 1 <sup>st</sup>           | Last                    | 1 <sup>st</sup>                              | Last                  | 1 <sup>st</sup>        | Last                   | 1 <sup>st</sup>         | Last                   | 1 <sup>st</sup>       | Last                  |
| 1 | 0.73 <sup>±0.38</sup>    | 0.60 <sup>±0.23</sup> | 2.67 <sup>±0.88</sup> | 2.50 <sup>±0.12</sup> | 13.33 <sup>±4.41</sup>    | 30.30 <sup>±3.10</sup>  | 1.92 <sup>±0.94</sup>                        | 0.41 <sup>±0.01</sup> | 98.57 <sup>±0.73</sup> | 99.50 <sup>±1.10</sup> | 21.57 <sup>±14.39</sup> | 12.50 <sup>±0.20</sup> | 7.67 <sup>±0.44</sup> | 7.80 <sup>±0.14</sup> |
| 2 | 0.90 <sup>±0.21</sup>    | 1.05 <sup>±0.75</sup> | 2.67 <sup>±0.33</sup> | 3.75 <sup>±0.13</sup> | 36.67 <sup>±8.82</sup>    | 35.00 <sup>±15.00</sup> | 0.46 <sup>±0.25</sup>                        | 0.39 <sup>±0.18</sup> | 99.23 <sup>±0.43</sup> | 98.30 <sup>±0.10</sup> | 4.70 <sup>±2.83</sup>   | 8.40 <sup>±7.50</sup>  | 7.83 <sup>±0.17</sup> | 7.75 <sup>±0.25</sup> |
| 3 | 0.75 <sup>±0.15</sup>    | 0.85 <sup>±0.35</sup> | 2.50 <sup>±0.50</sup> | 2.75 <sup>±0.75</sup> | 30.00 <sup>±10.00</sup>   | 22.50 <sup>±7.50</sup>  | 0.64 <sup>±0.10</sup>                        | 1.04 <sup>±0.45</sup> | 95.95 <sup>±0.95</sup> | 99.25 <sup>±0.25</sup> | 5.15 <sup>±1.05</sup>   | 2.90 <sup>±0.50</sup>  | 8.00 <sup>±0.1</sup>  | 8.00 <sup>±0.01</sup> |

Values (mean $\pm$ S.E.) within the same row bearing similar lowercase (small) letters and values within the same column bearing similar uppercase (capital) letters are not significantly different at  $p < 0.05$ .

Furthermore, a high level of epinephrine maintained during exhaustion test stimulates the conversion of muscle glycogen [17] to glucose phosphate. During exercise, enzymes produced (e.g. tissue lipase) stimulate glucose synthesis, hepatic glycogenolysis and lipolysis; these actions facilitate metabolism to provide additional fuel [16].

In shaded rams, the ejaculate volume (EV) was significantly ( $p < 0.05$ ) reduced throughout the three days POE (Table 6). This is clearly associated with the

continuous withdrawal of semen from the epididymal reserve and slow replacement by testicular, epididymal and accessory genital glands secretions. Similar findings were reported in frequently ejaculated Sudanese Desert [22], Konya Merino [23] and Santa Ines [24] rams. Frequent ejaculation of unshaded rams significantly reduced the EV of 1<sup>st</sup> day last collection compared to the other days (Table 7). This could be attributed to the effects of thermal stress and frequent ejaculation on the testes and accessory genital glands function.

The sperm individual motility (SIM) of shaded rams was significantly ( $p < 0.05$ ) lowered in the 1<sup>st</sup> and 3<sup>rd</sup> day POE (Table 6). This could be associated with the time needed for spermatozoa in the epididymal transit to acquire their ability of motility. Under normal conditions, the spermatozoa stay for about 16 days in the epididymis of rams [25]. It was reported that more than 3 days are required for the passage of spermatozoa in epididymal duct for frequently ejaculated rams [26, 27]. The reduction in SIM may also be related to insufficient supply of  $\text{Ca}^{2+}$  and Mg ions from epididymal duct fluid. In frequently ejaculated Konya rams, these cations were positively correlated with sperm SIM [23]. Other studies reported lower SIM in response to successive collection of semen [4, 22].

In the unshaded rams, the exhaustion test significantly ( $p < 0.05$ ) lowered the SIM of the last collection of the 3<sup>rd</sup> day of the experimental period (Table 7). This could be attributed to exhaustion of the epididymal sperms store. Also the reduction in sperm SIM could be attributed to the low availability of energy sources in seminal plasma which include fructose and adenosine triphosphate (ATP). The reduction in energy sources is related to the reduction in testicular, epididymal and accessory genital glands secretions and enzymatic activity [23, 28]. Similar reduction in SIM was reported in unshaded Hamari rams [1].

The significant ( $p < 0.05$ ) reduction in sperm cell concentration (SCC) observed in the first two days in shaded rams POE (Table 6) is clearly related to depletion of epididymal sperm content. The tail of the epididymis contains about 70% of the total number of spermatozoa compared to excurrent ducts. In rams, the seminiferous tubules cycle takes 10 days with different stages of spermatogenic wave; successive collection of semen with lower rate of replacement of spermatozoa may result in epididymal reserve depletion. Similar findings of reduced SCC in response to successive collection of semen were reported in Sudanese Desert rams [22] and Merino rams [9, 28]. Frequent ejaculation also decreased the SCC in Najdi [29], Konya Merino [23] and Santa Ines [24] rams.

In unshaded rams the SCC was significantly decreased in the 1<sup>st</sup> ( $p < 0.01$ ) and increased in the 3<sup>rd</sup> ( $p < 0.05$ ) day POE (Table 7). The reduction could be associated with interruption in spermatogenic cycle and consequently a reduction in the number of spermatozoa produced. Similar results were previously reported [30]. However, the increase in sperm cell concentration during exposure to heat stress could be attributed to the reduction in the EV. Similar reduction in SCC was observed in unshaded desert rams [1].

The present results indicate that the combined effects of exposure to solar radiation and exhaustion test significantly ( $p < 0.05$ ) reduced the living sperm percent (LSP) of the first collection in the 3<sup>rd</sup> day compared to the first two days and to the last collection (Table 7). The reduction in LSP could be related to the biochemical changes in spermatozoa, in addition to the influence of high ambient temperature which alters the scrotal thermoregulation mechanism. These findings are in agreement with what was reported in unshaded desert rams [1]. Kaya *et al.* (2002) reported that, frequent ejaculation of Konya Merino rams increased sperm membrane damage and release of Aspartate Amino Transaminase (ATA), Glutamic Pyruvic Transaminase (GPT) and Lactate Dehydrogenase (LDH) in seminal plasma. In frequently ejaculated Desert rams, low LSP was reported [22].

Frequent ejaculation of shaded rams significantly ( $p < 0.05$ ) reduced the percent of abnormal sperm (ABS) of the 1<sup>st</sup> collection in the last two days compared to the 1<sup>st</sup> day and the last collection of 1<sup>st</sup> day compared to the 1<sup>st</sup> collection (Table 6). This reduction is clearly related to the continuous withdrawal of the abnormal sperms. However, the significant ( $p < 0.05$ ) increase of ABS reported in the 2<sup>nd</sup> day last collection compared to the 1<sup>st</sup> collection could be associated with low calcium ions and inadequate epididymal maturation. It was reported [23] that calcium ion ( $\text{Ca}^{2+}$ ) is negatively correlated to abnormal sperms percent in frequently ejaculated Konya Merino rams. In frequently ejaculated Desert rams, the increase in ABS was attributed to inadequate epididymal maturation [22]. However, in unshaded rams, the significant ( $p < 0.05$ ) reduction in ABS in the last two days of the 1<sup>st</sup> collection compared to the 1<sup>st</sup> day and in the last collection of the 3<sup>rd</sup> day compared to the 1<sup>st</sup> collection (Table 7) could be attributed to the effect of frequent ejaculation. However, the significantly ( $p < 0.05$ ) higher ABS reported POE in the 2<sup>nd</sup> day of the experimental period could be related to the effects of frequent ejaculation and thermal stress. An increase in ABS of heat stressed desert rams was previously reported [1].

Frequent ejaculation of shaded rams was significantly ( $p < 0.05$ ) associated with increase in semen pH of the 1<sup>st</sup> collection of the last two days (Table 6). The increase in semen pH could be attributed to changes in pH of spermatozoa related to biochemical changes associated with frequent ejaculation [23, 30]. Also a low fructose content of frequently ejaculated semen could be responsible for high semen pH. In Desert rams, [22] reported an increase in seminal plasma and low fructose concentration in association with frequent ejaculation.

In the tropics, sheep are exposed continuously to the harsh environmental conditions which include inadequate forages and extreme thermal load. The cumulative effects of these conditions retard their reproductive performance. Desert rams have to combat unfavorable environmental conditions and perform mating throughout the day. This study revealed that frequent ejaculation and heat stress adversely affected semen and blood parameters of desert Hamari rams, which may reflect on their physiological activities, reproductive capabilities and the conception rate of females. Further studies are needed to evaluate the rate of conception in ewes inseminated with fresh or preserved semen frequently ejaculated from desert Hamari rams in different seasons.

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