Journal of Infertility and Reproductive Biology
Journal of Infertility and Reproductive Biology, 2015, Volume 3, Issue 1, Pages: 150-154

# The effects of seasons on the testicular parameters and epididymal sperm of the Iranian river buffalo (*Bubalus bubalis*)

**Saeed Moosa-Ali[1], Farid Barati[1*], Saleh Esmaeilzadeh[2], Dariush Gharibi[2], Mahmood Khaksary Mahabady[3]**

*1. Department of Clinical Science, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran*
*2. Department of pathobiological sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran*
*3. Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran*

**Abstract**

The aim of the present study was to evaluate the effects of different seasons on the morphometric testicular parameters and the respective epididymal sperm parameters. Total numbers of 104 scrota of pubertal buffalo bull (24-48 months) include their contents were provided from the local slaughterhouse. Scrotal circumference was measured on its place before dissection. Morphological parameters of testis and epididymis and epididymal sperm were analyzed. The results showed the highest scrotal circumference and testicular weight as well as retrieved sperm concentrations in spring. However, sperm tail and midpiece abnormalities were highest and the sperm progressive motility was the lowest during spring. During summer the lowest testicular weight and retrieved sperm concentration as well as low sperm progressive motility was considered. In conclusion, the results of the present study confirm direct influence of different seasons on the epididymal sperm and the testicular parameters of the Iranian river buffalo bull.

**Keywords:** Season, Iranian river buffalo, Epididyme, Sperm, Testicular morphometry

## 1. Introduction

The effects of environment on the male reproductive parameters in different species have been demonstrated. In the seasonal breeder animals, photoperiod is the important factor that regulates the sexual behavior. However, the other environmental factors, e.g., temperature and food quality, may affect the reproductive function in all species. Chronic heat stress significantly destructed the testicular parenchyma in ram (1) that raised the question about the effect of thermal stress in the tropical region on the buffalo bull fertility. Different studies have shown that sperm quality is influenced by season (2-5).

This effect of season on sperm quality may return to the changes within seminal plasma biochemical composition (6-9) or alteration in the testicular structure (10). However, the buffalo bull semen production is under influence of management and food availability and quality (11) rather than photoperiod. There is not any study on the seasonal changes of the epididymal sperm and testicular morphometric parameters in Iranian river buffalo. Therefore, the aim of the present study was to investigate the effects of different seasons on the seminal plasma-unexposed buffalo epididymal sperm.

[*]**Corresponding Address:** Dr. Farid Barati, Department of Clinical Science, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran. E-mail: fabrtir@yahoo.com

## 2. Materials and Methods

### 2.1. Materials

The Tyrod's solution ingredients (100 mM NaCl, 3.1 mM KCl, 25 mM $NaHCO_3$, 0.29 mM $NaH_2PO_4$ $H_2O$, 2.1 mM $CaCl_2$ $2H_2O$, 0.4 mM $MgCl_2$ $6H_2O$, (12), hematoxylin (H), eosin-Y (E) and nigrosin and sodium lactate were from Merck, Germany. Bovine albumin (BSA), sodium pyrovate and HEPES buffer

(10 mM) were bought from Sigma-Aldrich, USA. Tyrod- Albumin-Lactate -Pyrovate (TALP) was prepared with addition of BSA (0.006 g/ml), sodium pyrovate (1 mM) and sodium lactate (21.6 mM) to the Tyrod's solution.

**2.2. Study location**

The experiment was conducted from 1 February to 30 October 2012 at Ahvaz city in southwest 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.

**2.3. Sample collection and analysis**

In an incomplete randomized block design, total number of 104 pairs of the buffalo (Bubalus bubalis) testes-epididymides (35.7±1.01; 24-48 months; estimated based on the dental combination (13)) were collected within scrotum from the local slaughterhouse and transported to the laboratory on ice during four different seasons.

Scrotal circumference was measured on slaughtered animals before dissection, using a flexible plastic tape. The testes were removed and examined for morphological parameters (length, width and weights).

After gross examination, the cauda epididymis was dissected and incised on its ventral surface, then left within warmed TALP (2 mL) for 15 min. The retrieved sperm concentration was evaluated by placing a drop (1/20 dilution rate) of formalin diluted (3%) sperm sample on a modified Neubar slide. Sperm progressive motility was assessed by placing a drop of sperm suspension on a warm slide under a light microscopy with magnification of 400 and observation of three different microscopic fields each for 30 seconds.

Sperm was evaluated for vitality by placing a drop of sperm and mixing with equal volume of Eosin Y (0.69% w/v)/ Nigrosin (10% w/v) stains then counting pink sperms as dead.

Morphological abnormalities of sperm were evaluated by preparing methanol fixed smears of sperm and staining with conventional 20% Giemsa (Baharafshan, Iran) stain.

Morphologic abnormalities of head, midpiece and tail were evaluated under light microscopy and magnification of 1000. Only the samples with no microscopic lesion within testicular parenchyma were studied.

**2.4. Statistical analysis**

As the interaction of right or left side with seasons On the morphological parameters of testis and epididymis was non-significant, the effects of both factors on the parameters were analyzed separately. The effects of seasons on the testicular morphologic parameters and sperm parameters were analyzed using the General Linear Model procedure in SAS, and the least square means (L.S. means) were separated by Turkey's multiple range test (14).

## 3. Results

All of the sperm parameters and the testicular parameters were not significantly different between right and left testis (P>0.05).

Table 1 shows the effects of seasons on different sperm parameters and testicular morphologic parameters of the river buffalo. The mean retrieved sperm from testis was $179.3 \times 10^6$ sperm/mL that was highest in summer and lowest in autumn (P<0.0001).

Mean percentage of sperm motility was 30 that significantly changed over the seasons (P<0.0001). Significant decrease was observed in sperm progressive motility during spring and summer compared to autumn and winter. Sperm viability was estimated 96.8 %, that significantly differed among seasons (P<0.0001).

The lowest percentage of viable sperm was estimated in spring and autumn (Table 1).

Mean percentage of head, tail and midpiece abnormalities were 0.5, 6.9 and 1.3 that were significantly different among seasons (P<0.05).

The percentage of sperms with cytoplasmic droplet was 69.9 that reached the highest levels during summer and autumn (P<0.05).

The mean testicular length and width were 13±0.09 and 6.4±0.06 cm, respectively. These values were significantly changed over the seasons; maximum of the respected values were in spring and autumn.

The mean epididymal length was 16.4±0.16 cm, and the maximum value was recorded in autumn. The mean epididymal and testicular weights were 20.8±0.43 and 159±3.33 g. These parameters were also affected by season and the mean maximum values were in spring. Mean scrotal circumference was 30.3±0.31 cm that the highest value was recorded in spring.

Table 1. The epididymal sperm and some testicular parameters (LSmeans±SEM) of Iranian river buffalo (Bubalus bubalis)

| | Winter | Spring | Summer | Autumn | P value |
|---|---|---|---|---|---|
| Number of examined testes | 46 | 56 | 52 | 54 | |
| Count (×10$^6$/mL) | 123.1±64.1$^{a}$ | 297.7±22.84$^{b}$ | 121.4±25.63$^{a}$ | 111.12±23.4$^{a}$ | <0.0001 |
| Progressive Motility (%) | 58.7±8.7$^{a}$ | 15.4±3.12$^{b}$ | 13.9±3.38$^{b}$ | 55.3±3.18$^{a}$ | <0.0001 |
| Viability (%) | 98.6±0.83$^{a}$ | 96.3±0.34$^{b}$ | 98.3±0.33$^{a}$ | 95.9±0.91$^{b}$ | <0.0001 |
| **Abnormalities (%)** | | | | | |
| Head | 0.26±0.3$^{ab}$ | 0.51±0.12$^{ab}$ | 0.8±0.12$^{a}$ | 0.28±0.11$^{b}$ | 0.0135 |
| Tail | 3.3±1.75$^{a}$ | 11.6±0.62$^{b}$ | 3.5±0.71$^{a}$ | 5.3±0.63$^{a}$ | <0.0001 |
| Midpiece | 0.96±0.56$^{ab}$ | 2.09±0.20$^{a}$ | 0.86±0.23$^{b}$ | 0.88±0.21$^{b}$ | <0.0001 |
| Cytoplasmic droplet | 65.7±6.53$^{ab}$ | 60.3±2.33$^{a}$ | 76.4±2.64$^{b}$ | 75.4±2.64$^{b}$ | <0.0001 |
| Testicular width (cm) | 5.8±0.1$^{a}$ | 6.7±0.1$^{b}$ | 6.3±0.1$^{c}$ | 6.5±0.1$^{bc}$ | <0.0001 |
| Testicular length (cm) | 12.2±0.2$^{a}$ | 13.5±0.2$^{b}$ | 12.3±0.2$^{a}$ | 13.2±0.2$^{b}$ | <0.0001 |
| Epididymal length (cm) | 16.2±0.3$^{a}$ | 16.4±0.3$^{a}$ | 15±0.3$^{b}$ | 16.9±0.3$^{a}$ | <0.0001 |
| Epididymal weight (g) | 20.6±0.8$^{a}$ | 23.9±0.8$^{b}$ | 17.4±0.9$^{c}$ | 19.2±0.8$^{ca}$ | <0.0001 |
| Testicular weight (g) | 151.5±6.41$^{a}$ | 177.6±6.05$^{b}$ | 134.1±6.6$^{c}$ | 155±0.62$^{a}$ | <0.0001 |
| Number of examined animals | 23 | 28 | 26 | 27 | |
| Scrotal Circumference (cm) | 29.5±0.59$^{a}$ | 32.4±0.56$^{b}$ | 29.4±0.6$^{a}$ | 29.7±0.57$^{a}$ | 0.0004 |

$^{abc}$ values with a common letter within rows are not significantly different (P>0.05).

## 4. Discussion

The results of the present study clearly showed the significant effect of season on the testicular parameters and the respective epididymal sperm parameters of Iranian river buffalo. The testicular morphometric parameters and the concentration of retrieved sperm from epididymides were highest during the spring season in Iranian river buffalo. However, sperm progressive motility significantly reduced and the percentage of sperm tail and midpiece abnormalities were increased during this season. During summer, the lowest testicular weight and sperm progressive motility were considered. These results are in accordance with the previous studies that showed the satisfactory effect of spring season on the buffalo semen (9, 11). The effect of season on the reproductive parameters can be mediated by photoperiod, food quality and availability and temperature. Actually the buffalo species is not known as a seasonal breeder in term of photoperiodicity. Improving the testicular morphometric parameters in spring in the present study confirm that the buffalo bull reproduction depends on the nutritional status. However, reducing sperm progressive motility that was accompanied by the increasing sperm tail and midpiece abnormalities is an interesting and unpredicted finding during spring season in the present study. However, in line with results of the present study higher rate of the buffalo sperm tail abnormalities were reported during rainy season in Thailand (15).

The effect of season on the male reproduction may mediate thorough changes within the biochemical composition of seminal plasma or alteration of testicular structure. Nandre et al. (2013) showed that the chemical composition of seminal plasma during summer in the Indian river buffalo is different from that of winter sample and may contribute in lower semen quality of buffalo (9). Higher seminal plasma pH (16) and oesterogen concentration (7) and their correlation with the sperm parameters were reported in Nill-ravi buffalo bull during winter. However, seminal parameters of the swamp- buffaloes were not affected in term of seminal plasma pH, sperm concentrations (15). Asadpour et al (2007) and Sharma et al (2014) have shown a different protein expression within seminal plasma in Iranian river and Bhadawari buffalo bulls, respectively (4, 5). These changes can influence the quality of sperm during different seasons.

On the other hand, direct effect of chronic heat stress throughout the hot season on the ram sperm can be mediate by affecting the testicular paranchyma alterations (1) and has been reported in term of plasma membrane integrity (15) or DNA fragmentation (17) or susceptibility to cryopreservation damages (18) in buffalo bulls. The swamp-buffaloes in Thailand showed that sperm concentration is not affected by the season.

There are many studies on the scrotal circumference of buffalo breed in the world. A direct and positive correlation between age and weight of bull with scrotal circumference were reported in Murrah buffalo (19). To find the effect of the season, the pubertal buffalo bulls were selected for the present study. The previous reports on pubertal swamp buffaloes reported 17-20 cm (20) and 20-21 cm (21) that are less than the mean estimated scrotal circumference in the present study. The estimated value in the present study can be related to the breed difference and depends on the food availability and quality.

In summary, the highest value of testicular parameters of the Iranian river buffalo is in spring compared to the other seasons. However, the lowest sperm progressive motility and highest rate tail and midpiece abnormalities of the epididymal sperm during spring are noticeable and questionable in this breed in the region. During summer testicular parameters and the sperm progressive motility are in the lowest status. Some effects of season on the quality of buffalo bull sperm may not relate to alteration in seminal plasma biochemistry.

**Acknowledgements**

The authors thank to the research deputy of Shahid Chamran University for funding the study and acknowledge the office of Ahvaz city slaughterhouse for providing the samples.

**References**

1. Rasooli A, Taha Jalali M, Nouri M, et al. Effects of chronic heat stress on testicular structures, serum testosterone and cortisol concentrations in developing lambs. Anim Reprod Sci. 2010; 117(1-2): 55-59.
2. Andrabi S. Factors affecting the quality of cryopreserved buffalo (Bubalus bubalis) bull spermatozoa. Reprod Dom Anim. 2009; 44(3): 552-569.
3. Dixit NK, Agarwal SP, Agarwal VK, et al. Seasonal variations in serum levels of thyroid hormones and their relation with seminal quality and libido in buffalo bulls. Theriogenology. 1984; 22(5): 497-507.
4. Asadpour R, Alavi-Shoushtari SM, Rezaii SA, et al. SDS-polyacrylamide gel electrophoresis of buffalo bulls seminal plasma proteins and their relation with semen freezability. Anim Reprod Sci. 2007; 102(3-4): 308-313.
5. Sharma L, Pandey V, Nigam R, et al. Seasonal variations in seminal plasma proteins of Buffalo. Reprod Dom Anim 2014; In pressdoi: 10.1111/rda.12285.
6. Mandal DK, Nagpaul PK, Mohanty AK. Seasonal variation in hyaluronidase activity in seminal plasma of Murrah buffalo bulls in relation to seminal attributes and effect of thermal protection. Indian J Anim Sci. 2004; 74(10): 1007-1010.
7. Javed MT, Khan A, Ali M. Influence of season on seminal plasma testosterone and oestrogen in healthy and abnormal buffalo bulls and their relationship with other semen parameters. Vet Arhiv. 2000; 70(3): 141-149.
8. Khokhar BS, Singh M, Chaudhary KC. Transaminases in cattle and buffalo (Bubalus bubalis) semen in relation to fertility and seminal characteristics during moderate and colder seasons. Anim Reprod Sci. 1987; 13(3): 177-182.
9. Nandre RM, Fatima S, Bhupal G, et al. Assessment of variations in Indian Bubalus bubalis seminal plasma proteins during winter and summer seasons. Iranian J Vet Res. 2013; 14(1): 1-8.
10. Sharma AK, Gupta RC. Estimation of daily sperm production rate (DSPR) by quantitative testicular histology in Buffalo-bulls (Bubalus bubalis). Arch Androl. 1979; 3(2): 147-152.
11. Dahiya SS, Singh P. Nutritional and other management practices for optimum semen production in buffalo bulls. Buffalo Bulletin. 2013; 32(1): 277-284.
12. Parrish JJ, Susho-Parrish J, Winer MA, et al. Capacitation of bovine sperm by heparin. Biol Reprod. 1988; 38(5): 1171-1180.
13. Cockrill WR. Management, conservation and use. In W. R. Cockrill (Ed.). (1974)., The husbandry and health of the domestic buffalo (pp. 276-312). Rome, Italy: Food and Agricultural Organization.
14. SAS Institute Inc., SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2000-2004.
15. Koonjaenak S, Chanatinart V, Aiumlamai S, et al. Seasonal variation in semen quality of swamp buffalo bulls (Bubalus bubalis) in Thailand. Asian J Androl. 2007; 9(1): 92-101.
16. Javed MT, Khan A, Kausar R. Effect of age and season on some semen parameters of Nili-Ravi buffalo (Bubalus bubalis) bulls. Vet Arhiv. 2000; 70(2): 83-94.
17. Koonjaenak S, Rodriguez-Martinez H. Assessment of semen quality in Swamp Buffalo AI Bulls in Thailand. Italian J Anim Sci. 2007; 6(2): 701-704.
18. Koonjaenak S, Johannisson A, Pongpeng P, et al. Seasonal variation in nuclear DNA integrity of frozen-thawed spermatozoa from Thai AI swamp

buffaloes (Bubalus bubalis). J Vet Med Series A: Physiology Pathology Clinical Medicine. 2007; 54(7): 377-383.

19. da Luz PA, Santos PR, Andrighetto C, et al. The correlation between age, body weight and testicular parameters in Murrah buffalo bulls raised in Brazil. J Reprod Develop. 2013; 59(1): 14-17.
20. McCool CJ, Entwistle KW. The development of puberty and sexual maturity in the Australian Swamp buffalo bull. Theriogenology. 1989; 32(2): 171-184.
21. CJ MC, Entwistle KW Reproductive function in the Australian Swamp buffalo bull: Age effects and seasonal effects. Theriogenology. 1989; 31(3): 583-594.