

Reproductive Activity of Mature Iraqi Bull Buffaloes: Testes Dimensions and Histological Picture

N.S. Ibrahim, M.M.H. Al-sahaf and A.F. Alwan

Department of Surgery and Obstetrics, College of Veterinary Medicine, Baghdad University, Iraq

Abstract: The present study was carried out to investigate the reproductive activity of mature Iraqi bull buffaloes and effect of month's changes on the testes activity. Two hundred and fifty testes of mature buffaloes bull were taken from the slaughter house from December 2010 to September 2011. After slaughtering the length and width of the right testes were measured weight of the testes were also taken then carefully the epididymis were dissected. The diameters of testicular seminiferous tubules were also measured. The present study demonstrated a significant increased ($p < 0.05$) in weight (g), length and width (cm) in the testes in March and April. The diameter of the seminiferous tubules demonstrated higher ($p < 0.05$) significant in April, March and May, thickness of seminiferous tubules demonstrated higher significance in April, May and March. The increase in spermatogenesis process lead to increase the diameter and thickness of seminiferous. Conclusions reproductive activity of bull buffalo and semen physical characteristics decreased in hot months and increased in the moderate and cold months. The increase of ambient temperature in hot months lead to disturbance in reproductive activity but didn't stop it.

Keywords: Male buffaloes, seminiferous histology, seminiferous tubules, testes buffaloes

INTRODUCTION

Domestic water buffaloes are common in southern Iraq, Water Buffaloes are believed to originate in South Asia according to Cockrill (1993).

The buffalo has been traditionally regarded as a poor breeder with low reproductive efficiency, characterized by late attainment of puberty and maturity, seasonality of calving, long postpartum anoestrus, poor expression of estrous signs, low conception rates and long calving intervals (Perera, 1999). Seasonal reproductive pattern is also found and the inference from a series of studies is that seasonality is influenced by photoperiod, mediated by melatonin secretion (Zicarelli, 1997). In fact, the buffalo is a short-day breeder, becoming sexually active in response to decreasing day length in the late summer to early autumn (Arrighi *et al.*, 2010).

Buffalo bulls are capable of breeding throughout the year, but some seasonal fluctuation in reproductive function is evident in most countries where this species is reared (Sengupta *et al.*, 1963), with semen quality being poorer during summer than in winter. A morphological study of the testis is mandatory to evaluate the influence that different factors, such as hormonal fluctuations of the photo-neuroendocrine circuit may have on reproductive efficiency which may be a result of seasonal variation (Pant *et al.*, 2003; Yasuo *et al.*, 2006; Al-Sahaf and Ibrahim, 2012).

The aim of this investigation is to evaluate useful parameters to determine the relationship between gonadic physiology and reproductive variation during the period from December to September. A morpho- and histometric approach was used, as methods allow for objective and quantitative evaluations of semen.

MATERIALS AND METHODS

Two hundred fifty testes of healthy mature buffalo bulls, aged 3-6 years, were obtained from the slaughter house from December 2011 to September 2012, 3 visit/week. After slaughtering the right testes were collected and placed in a plastic box which contained ice until transporting within 2 h to the laboratory, the tissue which surrounds the testes and the epididymis was removed by scissors and scalpel. Testes weight was taken by using digital electrical balance (Sartorius, Germany) and length and width were measured by the (vernea). Histological sections were prepared according to Luna (1968). The diameters of the testicular seminiferous tubules were measured in 5 cross-sections per sample. Diameters of seminiferous tubules were measured by calculating horizontal and vertical diameters of the 5 tubules which were selected randomly for each sample by using Ocular micrometer when match with stage micrometer by different magnification then calculating the means of these diameters and the height of principal cells was

measured from the basement to the apical membrane in cross section of 5 tubules (Albert and William, 1981). Data were analyzed statistically by a complete randomized design in one-way ANOVA. Group differences were determined using Duncan Test at ($p \leq 0.05$) (Duncan, 1955; Steel and Torrie, 1980). In the statistical analyzed, (SPSS, 2008) was used. Group differences were determined using the Least Significant Difference (LSD) test at $p < 0.05$ (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

In present study the results of the testicular measurement showed months-related differences, even if accompanied by remarkable variations between individuals. The results of testicular weight are demonstrated in Table 1. The table showed that testicular weight increased ($p < 0.05$) significantly during January, April and March and decreased in September and August. Testicular length and width increased ($p < 0.05$) significantly in April, February and May then decreased in December and January, respectively. The increased of the weight, length and width in these months indicated an increase in the physiological activity of the testes in such months and an increased in activity of seminiferous tubules and sperm production in the testes like the morph metric measures of testes in different seasons that indicated an increase in mating seasons (late autumn-winter) and a decrease in non mating seasons, late spring to the beginning of autumn, (Arrighi *et al.*, 2010), this is similar to bull (Egbunike *et al.*, 1985; Alneamy, 1995; Hasan, 2005) and resemble studies on camel (Saheed, 1980).

An initial rise in Follicle Stimulating Hormone (FSH) results in a proliferation of Sertoli cells, a lengthening of the seminiferous tubules and an increase in tubule diameter. At the same time, there is a rise in Luteinizing Hormone (LH) secretion resulting in increased testosterone production by the Leydig cells (Albert and Leonardo, 2004). Weight of the testis was one of the markers of a possible alteration in androgen status, A decrease in testicular weight is most likely due to a decrease in the level of serum testosterone (Simanainen *et al.*, 2008).

The results of Table 2 demonstrated that diameter of seminiferous tubules ($p < 0.05$) increased significantly in April, March and May these increase in diameter of seminiferous tubules a companied by an increase in thickness of seminiferous tubules in the same months in April March and May. The histometric measures of testes showed that diameter of seminiferous tubules was decreased in hot months, in August, July and, September, this decrease in diameter of seminiferous

Table 1: Monthly changes in the weight in (g), length and width in (cm) of buffalo testes M+S.E.

Months	Weight (g)	Length (cm)	Width (cm)
December	99.32±1.78 ^{ab}	8.70±0.40 ^b	4.26±0.12 ^b
January	106.48±3.86 ^a	8.88±0.20 ^b	4.17±0.07 ^b
February	100.80±1.56 ^{ab}	9.44±0.23 ^a	4.38±0.12 ^{ab}
March	98.46±1.43 ^{ab}	9.17±0.20 ^{ab}	4.47±0.13 ^{ab}
April	105.36±1.23 ^a	9.69±0.17 ^a	4.62±0.11 ^a
May	96.08±3.78 ^b	9.38±0.17 ^a	4.53±0.08 ^a
June	99.80±0.77 ^{ab}	9.07±0.14 ^{ab}	4.43±0.07 ^{ab}
July	100.16±0.86 ^{ab}	9.27±0.15 ^{ab}	4.31±0.07 ^{ab}
August	96.20±1.07 ^b	9.13±0.16 ^{ab}	4.50±0.06 ^a
September	95.20±1.28 ^b	9.28±0.17 ^{ab}	4.42±0.09 ^{ab}

Small different letters that indicated a significant difference between months ($p < 0.05$)

Table 2: Shows diameter and thickness of seminiferous tubules of the testes (mille micron) (Mean±S.E.)

Months	Diameter of seminiferous tubules X40	Thickness of siminiferous tubules X40
December	233.45±4.04 ^c	54.11±1.15 ^c
January	235.26±2.30 ^c	56.13±2.31 ^c
February	258.24±4.61 ^{ab}	64.48±3.46 ^b
March	262.68±4.62 ^a	69.13±3.48 ^a
April	270.25±2.88 ^a	75.24±2.89 ^a
May	260.71±5.77 ^a	65.32±1.73 ^b
June	253.81±4.04 ^b	57.28±1.70 ^c
July	221.33±5.77 ^d	40.09±2.31 ^d
August	219.75±3.46 ^d	38.89±1.74 ^d
September	231.17±3.40 ^d	45.12±2.89 ^d

Small different letters that indicated a significant difference between months ($p < 0.05$)

tubules companied by a decrease in thickness of seminiferous tubules in the same months.

The results of this study demonstrated an increase in the activity of the testes in moderate and cold months and these activity is regulated by the increases testosterone hormone levels in these months (Tekepetery and Amman, 1989).

The increase in activity of testes corresponded with an increased of histological measurement of testes that increased significantly in moderate and cold months this increase in diameter and thickness affected by an increase in the spermatogenesis production and sperm concentration. The increases in spermatogenesis process lead to increase the diameter and thickness of seminiferous tubules, these results are in agreement with (Al-Jalaby, 1994) in goats. The seasons variation significantly effected the number of spermatogonia of seminiferous tubules in different stages that showed higher in spring and lower in summer (Hasan, 2005). The testes are affected by the increase of temperature lead to decreasing the number of the receptors of Luteinizing Hormone (LH) that presents on Leydig cells and then decreasing in testosterone hormone and activity of the testes in hot months (Wu and Murono, 1996; Al- Sahaf and Ibrahim, 2012).

The mean testicular weights and the mean diameter of the organ were higher especially in moderate months than in hot and cold months. Spermatogenesis was conserved in the resting period, but the test is showed smaller tubular diameters (Arrighi *et al.*, 2010) Seasonal variations of genitalia

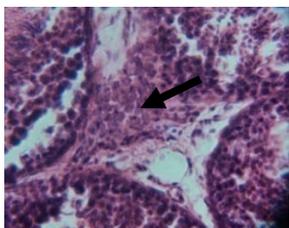


Fig. 1: Testes of buffalo in January shows moderate proliferation of Leydig cells between seminiferous tubules (→) (H and E 40X)

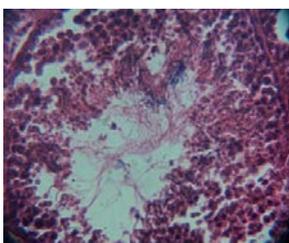


Fig. 2: Testes of buffalo in February shows moderate spermatogenesis and sperm in the lumina of seminiferous tubules (→) (H and E 40X)

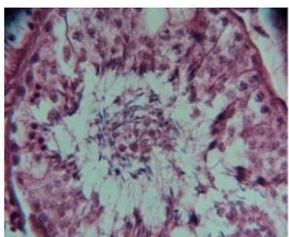


Fig. 3: Testes of buffalo in March shows marked spermatogenesis with spermatogenic cells filled most of seminiferous tubules (H and E 40X)

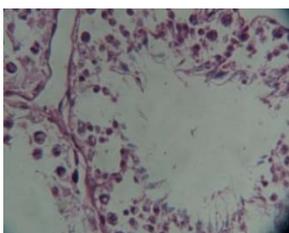


Fig. 4: Testes of buffalo in June shows decreased in spermatogenesis with absence of sperms in the lumina (H and E 40X)

were recorded in Iraqi bulls (Al-Neamy, 1995). Buffalo bulls are known to show seasonal rise and fall in reproductive functions, bull buffalo have capable of breeding throughout the year, which have been recorded in most countries where this species is reared (Rajamahendran and Sultan, 1987; Esposito *et al.*, 1992; Zicarelli, 1997).

In the current experiment, a comparison of buffalo male reproductive organs collected in mating and non-

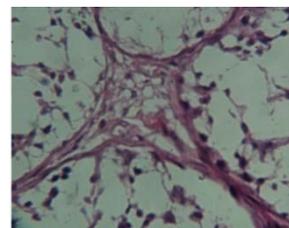


Fig. 5: Testes of buffalo in June shows few Leydig cells in the interstitial tissue with decreased in spermatogenesis (H and E 40X)

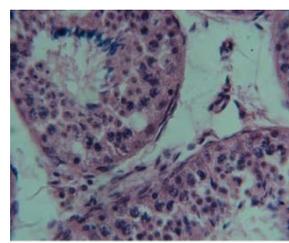


Fig. 6: Testes of buffalo in September complete process of spermatogenesis but moderate number and size of Leydig cell (H and E 10X)

mating seasons showed that morphological integrity was conserved either at testicular levels.

Smaller testicular volumes, together with minor values of tubular diameters might indicate a decrease in spermatogenesis (Al-Sahaf and Ibrahim, 2012).

The study revealed moderate morphological alteration between seasons. At winter it showed moderate spermatogenesis and sperm in the lumina of seminiferous tubules and moderate proliferation of Leydig cells in the interstitial tissues between seminiferous tubules as shown in Fig. 1 and 2. At spring, the main characteristics of histological lesions characterised by large size and proliferation of Leydig cells in the interstitial tissue, with marked spermatogenesis. The spermatogenic cells filled most of the seminiferous tubules lumina as well as sperm in their lumina Fig. 3. At summer, the histological section revealed a decrease in the spermatogenesis and the seminiferous tubules empty with few Leydig cell in the interstitial tissue as in Fig. 4 and 5. At autumn, the seminiferous tubules showed moderate proliferation in spermatogenesis with moderate number and size of Leydig cells Fig. 6. The variation changes in reproductive organs were noticed in most parts of the male reproductive tract in the roe deer (Goeritz *et al.*, 2003), demonstrating the interplay of the morphological state of all components of the reproductive tract and the production of spermatozoa, semen plasma and testosterone and in the bull by studying the seasonal variation on the reproductive system of bull (Al-Neami, 1995; Hasan, 2005). Also, in Murrah buffalo bulls, a significant positive relationship between scrotal circumference and semen volume and concentration per

ejaculate was reported (Pant *et al.*, 2003), indicating that in buffalo, as in cattle, scrotal circumference is a useful indicator of potential sperm output and may serve as an important criterion for selecting young bulls as Artificial Insemination sires. Present results demonstrated that the Iraqi buffaloes are not seasonal animals, because they are capable of being inseminated in around year but, the male reproductive organs effects by temperature in hot months (summer). The weight organs significantly of the male genital system decreased and led to disturbance in reproductive activity of the male. But it is a accompanied by disturbance of the male genital system in hot months (summer). As well as, the level of nutrition may affect on the reproductive activity of the animal.

REFERENCES

- Albert, B. and J.H. William, 1981. Diagnostic Procedure for Bacterial, Mycotic and Parasitic Infection. 6th Edn., American Public Health Association, Washington, DC., pp: 171-214.
- Albert, D. and F.C. Leonardo, 2004. Pubertal development of Bostaurus bulls. *Large Ani. Vet. Rounds.*, 4(4).
- Al-Jalaby, A.A.A., 1994. Seasonal changes in reproduction system of goat in Iraq. M.Sc. Thesis, Vet. College University of Baghdad, Iraq, pp: 27-46.
- Al-Neamy, I.M.K., 1995. Seasonal changes in reproductive system of bull in Iraq. M.Sc. Thesis, Vet. College University of Baghdad, pp: 22-38.
- Al-Sahaf, M.M.H. and N.S. Ibrahim, 2012. The effect of months on physical of sperms and morphological alterations in the testes and epididymis of Iraqi. *Iraqi J. Vet. Med.*, 36(2): 204-208.
- Arrighi, S., G. Bosi, D. Gropeptti and F. Cremonesi, 2010. Morpho-and histometric evaluation on the testis and epididymis in buffalo bulls during different reproductive seasons. *Open Anat. J.*, 2: 29-33.
- Cockrill, W.R., 1993. Developing water buffalo. *Buffalo J.*, 3(1-11): 48-60.
- Duncan, D.B., 1955. Multiple range and multiple f-test. *Biometrix*, 11: 1-24.
- Egbunike, C.N., V.A. Togun and E.A. Againg, 1985. *World Review of Animal Production*. 21: 11-17.
- Esposito, L., G. Campanile, R. DiPalo, R. Boni, C. DiMeo and L. Zicarelli, 1992. Seasonal reproductive failure in buffaloes bred in Italy. *Proceedings of the 12th International Congress, the Hague, The Netherlands, Anim. Reprod. (ICAR)*, 4: 2045-2047.
- Goeritz, F., M. Quest, A. Wagener, M. Fassbender, A. Broich *et al.*, 2003. Seasonal timing of sperm production in roe deer: Interrelationship among changes in ejaculate parameters, morphology and function of testis and accessory glands. *Theriogenology*, 59: 1487-1502.
- Hasan, W.M., 2005. Seasonal changes in reproductive system of bull in Iraq. M.Sc. Thesis, College of Technology, Musyeb, pp: 35-52.
- Luna, L.G., 1968. *Manual of Histology Staining Methods of Armed Forces Institute of Pathology*. 3rd Edn., McGraw-Hill Book Co., New York.
- Pant, H.C., R.K. Sharma and S.H. Patel, 2003. Testicular development and its relationship to semen production in Murrah buffalo bulls. *Theriog*, 60: 27-34.
- Perera, O.B.M., 1999. Reproduction in water buffalo: Comparative aspects and implications for management. *J. Reprod. Fertil. Suppl.*, 54: 157-168.
- Rajamahendran, R. and B. Sultan, 1987. Effect of climatic environment on libido and semen characteristics of buffalo bulls in Sri Lanka. *Phil. J. Vet. Anim. Sci.*, 9: 260.
- Saeed, A.M., 1980. Seasonal changes in reproductive system of camel. M.Sc. Thesis, College of Science, University of Baghdad.
- Sengupta, B.P., M.S. Misra and A. Roy, 1963. Climatic environment and reproductive behaviour of buffaloes: I. Effect of different seasons on various seminal attributes. *Indian J. Dairy Sci.*, 16: 150.
- Simanainen, U., K. McNamara, R.A. Davey, J.D. Zajac and D.J. Handelsman, 2008. Severe subfertility in mice with androgen receptor inactivation in sex accessory organs but not in testis. *Endocrinology*, 8: 149-330.
- SPSS, 2008. *Statistical Package for the Social Science Version 16 and 17 (win/mac/linux), Users Guide* Spss Inc., Chicago 3, usn. Retrieved from: <http://www.spss.com>.
- Steel, R.G. and J.H. Torrie, 1980. *Principles and Procedures of Statistics*. 2nd Edn., McGraw-Hill Book, Co., Inc., USA.
- Tekepetery, F.R. and R.P. Amman, 1989. Localization of androgen receptors in rams epididymal principle cell. *J. Reprod. Fert.*, 87: 31.
- Wu, N. and E.P. Muroño, 1996. Temperature and germ cellregulation of Leyding cell proliferation stimulated by sertoli cellsecreted mitogenic factor: Apossible role in cryptochidism. *Andrologio*, 28: 247-257.
- Yasuo, S., N. Nakao, S. Ohkura, M. Iigo, S. Hagiwara *et al.*, 2006. Long-day suppressed expression of type 2 deiodinase gene in the mediobasal hypothalamus of the Saanen goat, a short-day breeder: Implication for seasonal window of thyroid hormone action on reproductive neuroendocrine axis. *Endocrinology*, 147: 432-440.
- Zicarelli, L., 1997. Reproductive seasonality in buffalo. *Bubalus Bubalis.*, IV/97(Suppl): 29-52.