

## TESTICULAR BIOMETRY AND ITS CORRELATION WITH BODY WEIGHT AND SEMEN OUTPUT IN MURRAH BULL

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### ABSTRACT

The present study was conducted to find out testicular biometry and its correlation with body weight, scrotal circumference semen production. Semen sample were collected from six sexually mature Murrah buffalo bulls, aged 4 to 12 years, maintained in bull station of College of Veterinary Sciences and Animal Husbandry, Kumarganj, Faizabad, Uttar Pradesh, India. Biometrical evaluation was performed by caliper in all experimental bulls. The biometric testicular parameters (scrotal circumference, testicular length, testicular width and testicular volume) were analyzed. Present findings indicate strong correlations between scrotal circumference, testicular volume and body weight.

The body weight was significantly ( $P<0.01$ ) and positively correlated with scrotal circumference ( $r=0.98$ ), testicular volume ( $r=0.97$ ), sperm concentration ( $r=0.94$ ), concentration/ejaculate ( $r=0.64$ ), initial motility ( $r=0.90$ ), live count ( $r=0.89$ ), whereas it was significantly ( $P<0.01$ ) negatively correlated with total sperm abnormality ( $r=-0.79$ ). The overall mean ( $\pm$ SE) scrotal circumference of Murrah bulls were ranges between  $31.02\pm 0.39$  to  $46.33\pm 0.43$  cm. A significant ( $P<0.01$ ) variation in S.C. was reported among the bulls. The scrotal circumference was significantly ( $P<0.01$ ) and positively correlated

with body weight ( $r=0.98$ ), testicular volume ( $r=0.96$ ), testicular parenchyma ( $r=0.98$ ), sperm concentration ( $r=0.91$ ), concentration/ejaculate ( $r=0.63$ ).

**Keywords:** Murrah bulls, biometry, body weight, testes, sperm output

### INTRODUCTION

Buffalo, through their potential for producing milk, meat and draft power, contribute significantly to the agricultural economy of many developing countries including India. One of the major constraints in maximizing the production of buffalo is their inherent low reproductive efficiency. Proper bull selection is the most rapid way to make genetic improvements to the herd. Performance testing provides valuable information that can be used in selection of superior breeding animals.

Testicular biometry is an important component of monitoring the testis for normality and gauging potential sperm production (Paula *et al.*, 2001). Biometric parameters, such as scrotal circumference (S.C.), testicular weight (T.W.) and testicular length (T.L.), and testicular volume (T.V.) and testicular parenchyma (T.P.) are essential measurements in the andrological evaluation of a breeding animal. Among these parameters, S.C. is

used most often because it is easy to measure and displays a high correlation with body weight and reproductive capacity (libido), particularly sperm production (Brito *et al.*, 2004), while the biometric data related to S.C. helps to define the reproductive parameters for a species. S.C. alone should not be used for the selection of breeders, rather, a complete andrological evaluation (a breeding soundness examination), including an evaluation of semen quality, should be performed to certify the reproductive capacity of a male (Ohashi *et al.*, 2007). The biometric parameters of buffalo testicles can be established using comparative studies with other species. Investigations into the mammalian body and testicular biometrics are important for various aspects of reproduction; these studies help to characterize puberty and sexual maturity and enable inferences about spermatogenesis (Assis Neto *et al.*, 2003).

According to the Society for Theriogenology (SFT), bull breeding soundness examination comprises general and reproductive physical examination, scrotal circumference indexed for age, semen motility and sperm morphology examinations (Alexander, 2008). The information on body and testicular development has been well studied in dairy bulls (Coulter and Foote, 1977), beef bulls (Sosa *et al.*, 2002), and buffalo bulls (Ahmad *et al.*, 2010).

The aim of this study was to identify the reproductive characteristics of Murrah buffalo bulls using testicular biometric parameters and their correlations with body weight and semen output.

## MATERIALS AND METHODS

### Animals

The study was carried out during October 2014 to April 2015. A total of six adult sexually mature male Murrah buffalo bulls, aged 4 to 12 years were used. They were maintained in bull station of College of Veterinary Sciences and A.H., Kumarganj, Faizabad, U.P., India. All these buffalo bulls were in good health. They were maintained in nearly identical nutritional and managerial condition throughout the period of study. Biometrical evaluation was performed using caliper in all 6 Murrah bulls. Semen was also collected using artificial vagina from these 6 Murrah bulls. All the experimental animals were examined for general health status and the appearance of genitalia. The scrotal skin was observed for any kind of lesions. The testes were palpated and observed for their size, shape, free movement and position in the scrotum.

### Body weight and scrotal circumference measurements:

The body weight of each bull was recorded in Kilogram (kg) in the morning before meal. The weights were taken with a top loading balance.

Scrotal circumference was measured as per method recommended by the Society of Theriogenology (Ball *et al.*, 1983). The testes were first retracted into the lower part of the scrotum for measurement of scrotal circumference. To prevent separation of the two testes, the thumb and the fingers were placed on the sides rather than on the front or back of the scrotum. Then a measuring tape (scrotal tape) was looped and placed around the greatest diameter of the scrotum and pulled snugly so that the tape was firmly in contact with the entire circumference. Repeated measurements were done

and the mean of the measures was recorded to ensure the accuracy.

### **Testicular size measurements (*In vivo*)**

Testicular measurements were made while bulls were restrained in standing position. The testes were brought into the distal part of scrotum and the greatest testis length and width were measured with the help of a flexible measuring tape. Testicular volume was determined using the formula of Fields *et al.* (1979):  $VT = 2[(r^2) \times \pi \times L]$ , where  $r^2$  = testis width (radius),  $\pi$  = correction factor (3.14) and  $L$  = testis length.

### **Semen collection and evaluation**

Semen samples were collected from six buffalo bulls by using artificial vagina maintained at temperature between 38°C and 41°C. The semen was usually collected early in the morning, before feeding. Semen sample were collected twice a day and twice a week intervals from 6 buffalo-bulls. A total of two ejaculates were taken with a minimum interval of 30 minutes. Each semen sample was examined for routine semen parameters (volume, total sperm concentration, pH, mass motility, initial progressive motility, per cent live count, abnormal sperm as per standard methods described earlier (Salisbury *et al.*, 1985).

### **Sperm concentration per ml and initial progressive motility (percent)**

Concentration of spermatozoa (million/ml) in the neat semen was determined by the haemocytometer method adopting RBCs counting procedure (Salisbury *et al.*, 1985). A drop of semen was placed on a pre-warmed glass slide and covered with a cover slip. The percentage of motile spermatozoa was assessed subjectively at 37°C, using a heated stage, by viewing 5 to 6 fields

per slide with the aid of a closed-circuit television attached to a phase contrast microscope (40X). A spermatozoon that moved due to swimming, regardless of its speed, was considered as to be motile.

### **Percentage of live spermatozoa and abnormal spermatozoa**

The percentage of live and dead spermatozoa in fresh ejaculates as well as in pre freeze and cryopreserved semen was estimated by differential staining technique using Eosin-Nigrosin stain (Campbell *et al.*, 1953). The smears were prepared in duplicate after mixing a small drop of neat semen with four drops of stain on a clean grease free microscopic slide at 37°C. Hundred spermatozoa were counted under the oil immersion, objective (100X) of a phase contrast microscope for estimating the percentage of live (unstained) spermatozoa. The pinkish (eosinophilic) and partially stained spermatozoa were classified as dead.

The same slide made for live and dead count was also used for the morphological study of sperm to find out sperm abnormalities.

### **Sperm membrane integrity (percent)**

Hypo-osmotic swelling test (HOST) was performed after slight modification of the experiment carried out in human being (Jayendran *et al.*, 1984) to assess the functional integrity of the sperm tail membrane which gives idea of the spermatozoal fertilizing capacity *in vitro*. Sodium citrate (0.73 g; Merck) and fructose (1.351 g; Merck) were dissolved in 100 ml distilled H<sub>2</sub>O to prepare HOS solution (osmotic pressure ~150 mOsmol/kg) and maintained at 37°C for 5 minutes before use 0.1 ml of each semen sample was mixed with 0.9 of HOS solution and incubated at 37°C for

60 minutes. After incubation, place a small droplet on glass slide and put cover slip and Observation for swollen tail under high power magnification of phase contrast microscope. One hundred sperm were assessed for their swelling ability in HOS. The swollen sperm characterized by coiling of the tail were considered having an intact plasma membrane.

### Statistical analysis

Data were presented as mean and standard error of the mean (SEM). Analysis of variance (ANOVA) was used to assess differences among the bulls. When the F ratio was significant ( $P<0.05$ ). Descriptive analyses of the mean and standard deviation for each testicular biometric parameter were also performed with the Graph Pad Prism 5 software.

## RESULTS AND DISCUSSION

### Body weight and scrotal circumference

The overall mean ( $\pm$ SE) body weight (Kg) of Murrah bulls was observed in the range between  $454.5\pm 2.19$  to  $611.3\pm 3.04$  Kg. Average body weight of each bull is presented in Table 1. It differed significantly ( $P<0.01$ ) among the bulls. The body weight was significantly ( $P<0.01$ ) and positively correlated with scrotal circumference ( $r=0.98$ ), testicular volume ( $r=0.97$ ), sperm concentration ( $r=0.94$ ), concentration/ejaculate ( $r=0.64$ ), initial motility ( $r=0.90$ ), live count ( $r=0.89$ ) and HOS ( $r=0.80$ ) whereas it was significantly ( $P<0.01$ ) negatively correlated with total sperm abnormality ( $r=-0.79$ ).

Relationship of age to body weight and scrotal circumference in Murrah bulls is presented in Table 1. In general, both mean SC and body

weight increased ( $P<0.05$ ) in a curvilinear manner. The mean scrotal circumference was increase with body weight in ND5, ND9, ND7, ND2, ND1 and ND4 respectively.

For Nd5 the maximum SC was 46.33 cm and minimum was 31.02 cm for ND4 bull and other ND9, ND7, ND2 and ND1bulls 40.07cm, 37.97 cm 33.38 cm, and 32.6 cm, respectively. The correlation between body weight and scrotal circumference were 0.94, 0.94, 0.60, 0.94, 0.81 and 0.94 for ND1, ND2, ND4, ND5, ND7 and ND9 respectively. Mean SC followed the same pattern as that of body weight.

Similar findings were also observed by Pant *et al.* (2003) and lower than the observation reported by Asghar *et al.* (1985); Heuer *et al.* (1987) and Koonjaenak *et al.* (2007) but higher than those recorded by the Luz *et al.* (2012).

Body weight was highly ( $P<0.01$ ) positive correlated with testicular volume, scrotal circumference which is comparable to the findings of Silva *et al.* (1999) (Pig) and Caurot *et al.* (1970) (Ram and Cattle) but lower correlation with these parameters was observed by Luz *et al.* (2012). The strength of correlation obtained suggested that scrotal circumference and testicular volume are useful parameter for the selection of breeding bulls.

### Testicular volume

The testicular volume mean ( $\pm$ SE) of experimental bulls was recorded between  $700.03\pm 4.70$  to  $1796.0\pm 9.23$  cm<sup>3</sup>. The average testicular volume of each bull is given in Table 1. Lower T.V. was observed Pant *et al.* (2003) and Sequeira *et al.* (2007). The testicular volume differed significantly ( $P<0.01$ ) among experimental bulls.

Testicular volume of bulls was significantly ( $P<0.01$ ) and positively correlated with body weight

Table 1. Testicular biometry (Calliper and Ultrasound) and seminal attributes in fresh Murrah bulls.

Bull No	ND-1	ND-2	ND-4	ND-5	ND-7	ND-9	Pool
B.W. (Kg)	479.5 <sup>a</sup> ±2.51	481.8 <sup>cd</sup> ±3.05	454.5 <sup>f</sup> ±2.19	611.3 <sup>a</sup> ±3.04	511.7 <sup>a</sup> ±2.94	583.7 <sup>b</sup> ±1.94	520.4±9.78
S.C. (Cm)	32.60 <sup>a</sup> ±0.75	33.38 <sup>cd</sup> ±0.53	31.02 <sup>f</sup> ±0.39	46.33 <sup>a</sup> ±0.43	37.97 <sup>a</sup> ±0.37	40.07 <sup>b</sup> ±0.53	36.89±0.91
T.V. (Cm <sup>3</sup> )	932.4 <sup>c</sup> ±16.36	990.7 <sup>d</sup> ±14.68	700.3 <sup>f</sup> ±4.70	1796.0 <sup>a</sup> ±9.23	1379 <sup>bc</sup> ±7.71	1379 <sup>b</sup> ±7.71	1196±61.22
Conc. (M/ml)	1428 <sup>cd</sup> ±38.02	1406 <sup>de</sup> ±22.51	1233 <sup>f</sup> ±11.30	1748 <sup>a</sup> ±20.68	1476 <sup>c</sup> ±25.36	1619 <sup>b</sup> ±30.61	1485±25.87
Vol. (ml)	5.49±0.29	4.80±0.34	5.05±0.32	5.19±0.32	5.11±0.29	5.35±0.26	5.17±0.12
C/E. (M/ml)	7843 <sup>abc</sup> ±497.7	6970 <sup>cs</sup> ±585.8	6239 <sup>def</sup> ±427.2	9101 <sup>a</sup> ±640.3	7552 <sup>bcd</sup> ±570.4	8685 <sup>ab</sup> ±504.3	7731±252.5
IM (%)	55.63 <sup>a</sup> ±0.42	58.25 <sup>d</sup> ±0.49	53.13 <sup>f</sup> ±0.64	66.88 <sup>a</sup> ±0.61	60.00 <sup>a</sup> ±0.65	63.25 <sup>b</sup> ±0.53	59.52±0.70
LC (%)	79.00 <sup>a</sup> ±0.71	83.00 <sup>d</sup> ±0.85	73.25 <sup>f</sup> ±0.88	89.63 <sup>a</sup> ±0.42	84.63 <sup>c</sup> ±0.57	87.25 <sup>b</sup> ±0.53	82.79±0.83
Ab. (%)	14.75 <sup>b</sup> ±0.31	14.00 <sup>bc</sup> ±0.46	16.88 <sup>a</sup> ±0.52	11.63 <sup>f</sup> ±0.26	13.38 <sup>cd</sup> ±0.18	12.88 <sup>de</sup> ±0.35	13.92±0.28
HOS (%)	34.00 <sup>a</sup> ±0.60	39.00 <sup>cd</sup> ±0.46	31.75 <sup>e</sup> ±0.73	43.25 <sup>a</sup> ±0.59	40.88 <sup>abc</sup> ±0.72	42.00 <sup>ab</sup> ±0.57	38.48±0.66

Mean bearing different superscript (a, b, c, d, e, f) in a column differed significantly ( $P < 0.05$ ), separately for each attributes, B.W= Body weight, S.C.= Scrotal circumference, T.V.=Testicular volume, Conc.=Concentration, Vol.=Volume, C/E.=Concentration/Ejaculate, IM=Initial motility, LC=Live count, Ab.=Abnormality, HOS= Hypo-osmotic swelling

( $r=0.97$ ), concentration ( $r=0.90$ ), concentration/ejaculate ( $r=0.55$ ), initial motility ( $r=0.89$ ), live count ( $r=0.88$ ) and HOS reactive spermatozoa ( $r=0.81$ ) whereas, significantly ( $P<0.01$ ) negatively correlation with total sperm abnormality ( $r=-0.81$ ). The findings of present study on correlation between various testicular indices were in agreement with the earlier study carried out on Murrah bulls. (Pant *et al.*, 2003).

### Semen quality

Relationship of age to semen quality (ejaculate volume, percent motility, sperm concentration, plasma membrane integrity, live sperm, and sperm abnormalities) is presented in Table 1. Average ejaculated volume (mean $\pm$ SE) of semen was observed in the range between 4.80 $\pm$ 0.34 to 5.49 $\pm$ 0.29 ml. Volume of semen was differed significantly ( $P<0.01$ ) between the ejaculate of same bull as well as among all experimental Murrah bulls. The mean value of ejaculate volume was comparable to those reported by Pillai, (1965); Dharmi, (1992); Srivastava, (2011) and Maurya *et al.* (2013).

The average sperm concentration of (million/ml) of Murrah buffalo bulls were recorded between 1233 $\pm$ 11.30 to 1748 $\pm$ 20.68. The present finding regarding sperm concentration ( $10^6$ /ml) was similar to the observation of Patil, (1981) and higher than those reported by Pillai, (1965); Kumar *et al.* (1993); Gokhale and Bhatt *et al.* (1996); Bhakat *et al.* (2011) and Srivastava, (2011) but lower than those reported by Bhakat *et al.* (2015). The wide variation in the sperm concentration has been attributed to factors like season, individuality, age of bull, sexual excitement frequency of collection etc. (Tomar, 1986).

The average per cent initial progressive motility, live spermatozoon, sperm abnormality

and HOS reactive spermatozoa in semen of Murrah buffalo bull under experimental condition was ranges between 53.13 $\pm$ 0.64 to 66.88 $\pm$ 0.61 %, 73.25 $\pm$ 0.88 and 89.63 $\pm$ 0.42, 11.63 $\pm$ 0.26 to 16.88 $\pm$ 0.52 % and 31.75 $\pm$ 0.73 to 43.25 $\pm$ 0.59, respectively.

The correlation of 0.96 between scrotal circumference and testicular volume is similar to the values (0.92 to 0.97) reported in several studies (Hahn *et al.*, 1969; Van Demark, 1986; Pant *et al.*, 2003; Gober *et al.*, 1998), whereas, positively lower correlation reported by Luz *et al.* (2012). The correlation between scrotal circumference and sperm output was recorded higher in all the bulls.

Others have reported similar trends in dairy bulls (Willet and Ohms, 1957; Hahn *et al.*, 1969 and Dhage *et al.*, 2010). These results emphasis the importance of scrotal circumference in selecting breeding bull for future semen production.

In conclusion, Higher correlation of body weight with testicular biometry and seminal attributes clearly indicate that higher body condition score may directly affect the performance of breeding bulls.

## REFERENCES

- Ahmad, N., S. Umair, M. Shahab and M. Arslan. 2010. Testicular development and establishment of spermatogenesis in Nili-Ravi. *Theriogenology*, **73**(1): 20-25.
- Alexander, J.H. 2008. Bull breeding soundness evaluation: A practitioner's perspective. *Theriogenology*, **70**: 469-472.
- Asghar, A.A., M.A. Chaudhry and J. Iqbal. 1985. Productive and reproductive performance of Nilli-Ravi buffaloes under optimal feeding and management conditions. *Sixth Annual*

- Report (1984-85)*. LPRI Bahadurnagar, Okara, Pakistan.
- Assis Neto, A.C., M.A.M. Carvalho, M.I.V. Melo, M.A. Miglino, M.F. Oliveira, M.M. Almeida, P.C. Papa, Jr.J.R. Kfoury. 2003. Biometric aspects of the testicular and corporal development of Agoutis (*Dasyprocta aguti*) raised in captivity. *Braz. J. Vet. Res. Anim. Sci.*, **40**: 154-160.
- Ball, A.B. 2008. Diagnostic methods for evaluation of stallion subfertility: a review. *J. Equine Vet. Sci.*, **28**: 650-664.
- Bhakat, M., T.K. Mohanty, V.S. Raina, A.K. Gupta and H.M. Khan. 2011. Frozen semen production performance of Murrah buffalo bulls. *Buffalo Bull.*, **30**(2): 157-162.
- Bhakat, M., T.K. Mohanty, S. Singh, A.K. Gupta, A.K. Chakarvarty and P. Singh. 2015. Influence of semen collector on semen characteristics of Murrah buffalo and Crossbred bulls. *Advances in Animal and Veterinary Sciences*, **3**(4): 253-258.
- Brito, L.F., A.E. Silva, M.M. Unanian, M.A. Dode, R.T. Barbosa, J.P. Kastelic. 2004. Sexual development in early and late maturing *Bos indicus* and *Bos indicus* x *Bos taurus* crossbred bulls in Brazil. *Theriogenology*, **62**: 1198-1217.
- Campbell, R.G., J.L. Hancock and L. Rothschild. 1953. Counting live and dead spermatozoa. *J. Expt. Biol.*, **30**: 44.
- Coulter, G.H. and R.H. Foote. 1977. Relationship of body weight to testicular size and consistency in growing Holstein bulls. *J. Anim. Sci.*, **44**: 1076-1079.
- Courot, M., M.T. Hochereau-De Reviers and R. Ortavant. 1970. Spermatogenesis. p. 339-432. In Johnson A.D., W.R. Gomes and N.L. Vandemark. (eds.). *The Testis*. New York, Academic Press.
- Dhami, A.J. 1992. *Comparative evaluation of certain procedures in deep freezing of cattle and buffalo semen under tropical climate*. Ph.D. Thesis. Indian Veterinary Research Institute, Izatnagar.
- Fields, M.J., W.E. Burns and A.C. Warnick. 1979. Age, season and breed effects on testicular volume and semen traits in young beef bulls. *J. Anim. Sci.*, **48**: 1299-1304.
- Gabor, G., R.G. Sasser, J.P. Kastelic, M. Mezes, G. Falkay, S. Bozo, J.C. Vrlgyi, I. Bárány, A. Hides, J.F. Szfisz and G. Borest. 1998. Computer analysis of video and ultrasonographic images for evaluation of bull testes. *Theriogenology*, **50**: 223-228.
- Gokhale, S.B., M. Mushtaque, N.L. Phadke and G.S. Ambhore. 2003. Studies on the effect of hydrogen ion concentration of extender on semen characters of Murrah buffalo bulls. *Indian J. Anim. Reprod.*, **24**(2): 158-160.
- Hahn, J., R.H. Foote and Jr.G.F. Seidel. 1969. Testicular growth and related sperm output in dairy bulls. *J. Dairy Sci.*, **29**: 41-47.
- Heuer, C., M.N. Tahir and H. Amjad. 1987. Effect of season on fertility of frozen buffalo semen. *Anim. Reprod. Sci.*, **13**: 15-21.
- Jeyendran, R.S., H.H. Vander-Ven, M. Perez-Pelaez, B.G. Crabo and L.J.D. Zanevld. 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characters. *J. Reprod. Fertil.*, **70**: 219-228.
- Jeyendran, R.S., H.H. Vander-Ven, M. Perez-Pelaez, B.G. Crabo and L.J.D. Zanevld. 1984. Development of an assay to assess the functional integrity of the human sperm

- membrane and its relationship to other semen characters. *J. Reprod. Fertil.*, **70**: 219-228.
- Koonjaenak, S., V. Chanatinart, S. Aiumlamai, T. Pinyopumontr and H. Rodriguez-Martinez. 2007. Seasonal variation in semen quality of swamp buffalo bulls (*Bubalus bubalis*) in Thailand. *Asian J. Androl.*, **9**: 92-101.
- Kumar, S., K.L. Sahni and G.S. Bistha. 1993. Cytomorphological characteristics of motile and static semen of buffalo bulls. *Buffalo Journal*, **9**(2): 117-127.
- Luz, P.A.C., P.R.S. Santos, S. Andrighetto, A.M. Jorge and A.C. Assis-Neto. 2012. The Correlation between Age, Body Weight and Testicular Parameters in Murrah Buffalo Bulls Raised in Brazil. *J. Reprod. Develop.*, **59**(1): 14-17.
- Maurya, K., O.P. Singh and S. Srivastava. 2013. Seminal plasma ascorbic acid level and its relationship to sperm characteristics in Murrah buffalo bulls. *Indian J. Anim. Sci.*, **83**(5): 498-501.
- Ohashi, O.M., M.S. Miranda, M.S. Cordeiro, S.S. Socorro and D. Santos. 2007. Reproductive development of male buffalo: testicular biometry, espermatic activity and endocrinology. *Rev. Bras. Repr. Anim.*, **31**: 299-306.
- Pant, H.C., R.K. Sharma, S.H. Patel, H.R. Shukla, A.K. Mittal and J.R. Kasira. 2003. Testicular development and its relationship to semen production in Murrah buffalo bulls. *Theriogenology*, **60**: 27-34.
- Patil, D.S. 1981. *Deep freezing of buffalo bull semen in various diluters*. M.V.Sc. Thesis. Kokan Krishi Vidyaapeeth, Dapoli, India.
- Paula, T.A.R and R.D. Navarro. 2001. Testicular componentes of peccaries (*Tayassu pecari*) and collared peccary (*Tayassu tajacu*). *Rev. Bras. Reprod. Anim.*, **25**: 206-207.
- Pilai, K.V. 1965. *Observation on certain semen characteristics of Murrha buffalo bulls*. M.V.Sc. Thesis, Bombay University, Bombay.
- Salisbury, G.W., N.K. VanDemark and J.R. Lodge. 1985. *Semen Evaluation, Physiology of Reproduction and Artificial Insemination of Cattle*, 2<sup>nd</sup> ed. CBS Publishers and Distributors, 485, Shahdara, Delhi, India.
- Silva, S.M., M.I.V. Melo, I.R. Scheid, E.F. Nascimento and G.D. Cassali. 1999. Establishment of spermatogenesis in pigs of Large White and Landrace breeds with different growth with emphasis on puberty. p. 301-302. *In Congresso Brasileiro de Veterinários Especialistas em Suínos*, Belo Horizonte, Brazil.
- Siqueira1, J.B., E. Oba1, R.O. Pinho, E.F. Castilho, R.F. Bittencourt, S.S. Assaf1 and J.D. Guimarães. 2007. Gonadic Sperm Reserve in Buffaloes Created under Tropical Conditions. *Ital. J. Anim. Sci.*, **6**(2): 615-618.
- Sosa, J.M., P.L. Senger and J.J. Reeves. 2002. Evaluation of American Wagyu sires for scrotal circumference by age and body weight. *J. Anim. Sci.*, **80**: 19-22.
- Srivastava, S. 2011. *Effect of Amino acids incorporation of post thaw seminal attributes and in vitro fertility assay of Murrah bull spermatozoa*. Ph.D. Thesis, Dr. Bhim Rao Ambedkar University, Agra, Uttra Pradesh, India.
- The Society for Theriogenology. 1983. *Manual for Breeding Soundness Examination of Bulls*. 12p.
- Tomar, N., 1986. *Artificial Insemination and*

*Reproduction of Cattle and Buffaloes*, 3<sup>rd</sup>  
ed. Saroj Prakashan Allahabad, India.

Van Der Ven, H.H., R.S Jeyendran, S. Al-Hasani,  
M. Perez-Pelaez, K. Diedrich and L.J.D.  
Zanaveld. 1986. Correlation between  
human sperm swelling in hypoosmotic  
medium (hypoosmotic swelling test) and *in*  
*vitro* fertilization. *J. Androl.*, **7**: 190-196.

Willet, E.L. and J.I. Ohm. 1957. Measurement  
of testicular size and its relation to the  
production of spermatozoa by buck. *J.*  
*Dairy Sci.* **40**: 1559-1569.