RESEARCH ARTICLE

Testicular Biometry, Body-weight, Seminal Attributes and their Correlations in Murrah Bulls

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Abstract

The present study was carried out to evaluate the testicular markers and their correlations with body-weight and seminal output in Murrah bulls. Semen samples were collected from eight Murrah buffalo bulls, aged 4 to 8 years, maintained at the bull station of deep-frozen semen lab of Veterinary College, Faizabad (Uttar Pradesh), India. Biometrical (scrotal circumference, testicular length, testicular diameter, and testicular volume) evaluation was performed by Vernier caliper and scrotal tape in all experimental bulls. The overall mean (± SE) body-weight, scrotal circumference, right testicular length, left testicular length, right testicular diameter, left testicular diameter, pooled testicular volume, ejaculate volume, sperm concentration, and sperm count per ejaculate of Murrah bulls were 716.5 ± 0.59 kg, 34.37 ± 0.41 cm, 11.85 ± 0.12 cm, 12.32 ± 0.14 cm, 7.81 ± 0.07 cm, 8.26 ± 0.08 cm, 791.4 ± 22.62 cm^3, 3.82 ± 0.12 mL, 1271 ± 22.68 million/mL, and 4852 ± 166.3 million with significant differences ($p < 0.01$) among the bulls. Left testis was significantly larger than the right one. There were strong correlations between scrotal circumference, testicular length, testicular diameter, testicular volume, and body-weight. The scrotal circumference was significantly ($p < 0.01$) and positively correlated with body-weight ($r = 0.88$), right testicular length ($r=0.79$), left testicular length ($r=0.81$), right testicular diameter ($r = 0.66$), left testicular diameter ($r = 0.75$), testicular volume ($r = 0.79$), and had weak correlations ($p < 0.05$) with sperm count ($r=0.25$) and ejaculate volume ($r = 0.25$). The testicular volume was significantly ($p < 0.01$) and positively correlated with body-weight, ejaculate volume, and total sperm output.

Keywords: Body-weight, Ejaculate volume, Murrah bull, Sperm output. Testes, Testicular volume.

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Introduction

Murrah, one of the dairy buffalo breeds in the largest numbers, is used for upgrading local/non-descript buffalo in India and elsewhere (Ahirwar *et al.*, 2018). Testicular markers *viz*., the testicular length (TL), testicular width (TWD), testicular thickness (TT), testicular weight (TW), and scrotal circumference (SC) are some essential parameters for monitoring the testis normality and judging the sperm production capacity (Paula and Navarro, 2001). Many workers have reported a significant positive correlation between scrotal circumference and body-weight with the advancement of age in Murrah buffalo (Patricia *et al*., 2013), swamp buffalo (Viana *et al*., 2008), and in Egyptian water buffalo bulls (Genedy *et al*., 2019). The biometric data related to testicular parameters and SC help in selecting breeding bulls and assist in reproduction to characterize puberty and sexual maturity and enable inferences about spermatogenesis (Patricia *et al*., 2013). Scrotal circumference alone should not be used for the selection of breeders. Instead, a complete andrological evaluation, including an evaluation of semen quality, should be performed to certify the reproductive capacity of a male (Ohashi *et al*., 2007).

Scrotal circumference is a crucial testicular parameter, easy to measure, and the most accurate indicator of semen quality (Pant *et al*., 2003). Testicular size is directly related to the total mass of sperm-producing tissues and the onset of puberty in bulls (Ashwood, 2009). The consistent increase in scrotal circumference improves the seminal attributes due to the increase in the total mass of sperm-producing tissues and the number of secretary tissues. There is a positive relationship of the scrotal circumference (SC) with the volume of ejaculate and the percentage of live sperm (Pant *et al*., 2003). The bulls having higher scrotal surface temperature gradient, lower testicular coverings, and higher scrotal circumference produced better quality semen (Kushwaha *et al*., 2018). The testicular volume was significantly and positively correlated with body-weight, sperm concentration/mL, and total sperm output/ejaculate in Murrah bulls (Srivastava, 2011; Kumar and

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Srivastava, 2017) and in bucks (Patel *et al*., 2021). This study aimed to determine testicular biometry and seminal output and their correlations in Murrah bulls.

Materials And Methods

The study was carried out from January 2020 to March 2021 on eight Murrah bulls of the age group 4-8 years managed identically at Deep Frozen Semen Lab, College of Veterinary Sciences, ANDUAT, Kumarganj, Ayodhya (Uttar Pradesh).

Measurement of Body-Weight, Scrotal Circumference, and Testis Size

The body-weight of each bull was recorded in kilogram with a top loading balance in the morning before feeding and watering. The scrotal circumference was measured as per the method recommended by the Society of Theriogenology (Ball *et al.,* 1983) after retracting the testes into the lower part of the scrotum.

Bulls were restrained and testicular measurements were made in standing position. The testes were brought into the distal part of the scrotum, and the greatest testis length and width were measured with the help of a Vernier caliper. Testicular volume was measured using the formula TV= TD^2xTLx0.5.

Characteristics of Semen

Semen was collected from 8 Murrah buffalo bulls twice in a week for quality and fertlity analysis using an artificial vagina maintained at temperature between 40 and 42^0C. Forty-eight ejaculates (6 from each bull) were collected early in the morning, before feeding, and each collection consisted of two ejaculates taken within 30 minutes. The characteristics of semen examined were ejaculate volume, sperm concentration/mL and total sperm output/ejaculate, The data were analysed statistically using one way ANOVA and Duncan's NMRT, and the Pearson's correlation coefficients among various attributes were determined.

Results And Discussion

Body-weight

The overall mean (± SE) body-weight of Murrah bulls was 716.00 ± 10.59 kg, and it ranged between 562 ± 4.79 and 794.2 ± 1.54 kg with significant ($p < 0.01$) differences among the bulls (Table 1). This variation among the bulls might be due to differences in their age. Similar findings were also observed by Luz *et al.* (2013) and Kumar and Srivastava (2017). The body-weight was significantly ($p < 0.01$) and positively correlated with scrotal circumference (=0.88), right testicular length (r=0.67), left testicular length (r=0.71), right testicular diameter (r=0.67), left testicular diameter (r=0.75) and testicular volume (r=0.73) (Table 2). These correlations were similar to the findings of Pant *et al.* (2003), Srivastava (2011), Luz *et al.* (2013), and Kumar (2014) in Murrah bulls. The strength of positive correlations obtained suggested that the scrotal circumference, testicular length, testicular diameter, testicular volume are useful parameters for the selection of breeding bulls. A higher correlation of body-weight with testicular biometry and a lower correlation with seminal attributes indicate that higher body conditions may directly affect the performance of breeding bulls.

Scrotal Circumference

The overall mean (± SE) scrotal circumference of Murrah bulls was observed to be 34.37 ± 0.41 cm, and it varied significantly ($p < 0.01$) between bulls from 30.13 ± 0.11 to 38.93 ± 0.10 cm (Table 1). This variation might be due to differences in the age of bulls. The mean value of scrotal circumference in the present study was similar to the observation recorded by Younis *et al.* (2003), however several other workers reported comparatively lower values of SC (Pant *et al.,* 2003; Luz *et al.,* 2013; Shende *et al.,* 2019).

The scrotal circumference was significantly ($p < 0.01$) and positively correlated with right testicular length (r=0.79), left testicular length (r= 0.81), right testicular diameter (r=0.66), left testicular diameter (r=0.75), and testicular volume (r=0.79), and it had weak correlations ($p < 0.05$) with volume of semen (r=0.25) and sperm output/ejaculate (r=0.25) (Table 2). These correlations were similar to the observations of Pant *et al.* (2003), Srivastava (2011), and Kumar (2014), whereas positive lower correlations were reported by Luz *et al.* (2013). These results emphasize the importance of scrotal circumference in selecting breeding bulls.

Testicular Length

In the present study, the length of left testis was higher (13.62 ± 0.07 cm) than right testis (11.85 ± 0.12) in all bulls, which differed significantly ($p < 0.01$) among the bulls (Table 1). Similar findings were also observed by Shende *et al.* (2019) and Kumar and Srivastava (2017) but were higher than those reported by Luz *et al.* (2013) and Genedy *et al*. (2019).

Right and left testicular length was highly ($p < 0.01$) positively correlated with scrotal circumference (r= 0.79 & =0.81). Shende *et al*. (2019) observed a similarly high correlation but lower than that of Pant *et al.* (2003) in Murrah bulls. Right and left testicular length were positively ($p < 0.01$) correlated with right (r= 0.88, 0.89) and left (r=0.90, 0.91 resp.) testicular volume (Table 2) as was recorded by Pant *et al.* (2003) in Murrah bulls.

Testicular Diameter

The overall mean (± SE) diameter of the right and left testis of Murrah bulls was recorded as 7.81 ± 0.07 and 8.26 ± 0.08 cm, respectively. The right and left testicular diameter differed significantly ($p < 0.05$) among the bulls (Table 1). A nearly similar finding was also recorded by Saurabh *et al.* (2018) but was higher than that reported by Luz *et al.* (2013). The right testicular diameter was positively correlated with right testicular volume (r=0.93) and left testicular volume

Table 1: Testicular biometry and seminal attributes of Murrah bulls (Mean ± SE)

Bull/ Para-meters	*BWt (kg)*	*SC (cm)*	*RTL (cm)*	*LTL (cm)*	*RTD (cm)*	*LTD (cm)*	*RTV (cm^3)*	*LTV (cm^3)*	*PTV (cm^3)*	*EV (ml)*	*Sp Conc. (million / mL)*	*Totol Sp. Conc./E (million)*
M-1	686.7 ± 3.33^{c}	33.63 ± 0.14^{d}	11.53 ± 0.09^{c}	11.82 ± 0.07^{b}	8.21 ± 0.05^{e}	8.58 ± 0.04^{c}	389.2 ± 7.18^{c}	434.7 ± 5.51^{d}	823.9 ± 11.66^{d}	3.72 ± 0.21	1412 ± 38.61^{b}	5222 ± 238.1^{b}
M-2	562.5 ± 4.79^{a}	30.13 ± 0.11^{a}	11.30 ± 0.06^{b}	11.47 ± 0.06^{a}	7.01 ± 0.07^{a}	7.29 ± 0.08^{a}	277.9 ± 7.21^{a}	305.1 ± 8.87^{a}.7	583 ± 15.27^{a}	4.10 ± 0.41	1335 ± 53.88^{b}	5375 ± 368.8^{b}
M-3	721.7 ± 3.07^{d}	31.65 ± 0.08^{b}	11.75 ± 0.04^{d}	12.18 ± 0.04^{d}	7.82 ± 0.07^{b}	8.24 ± 0.03^{b}	372.8 ± 10.89^{c}	401.2 ± 12.99^{cd}	773.9 ± 5.07^{c}	3.22 ± 0.14	1356 ± 51.97^{b}	4344 ± 188.9^{ab}
M-4	745.8 ± 2.01^{e}	34.97 ± 0.04^{e}	11.70 ± 0.05^{cd}	12.10 ± 0.04^{cd}	7.84 ± 0.02^{bd}	8.21 ± 0.05^{b}	359.6 ± 2.01^{c}	408.00 ± 5.163^{cd}	767.5 ± 6.06^{c}	3.53 ± 0.11	1075 ± 54.04^{a}	3786 ± 166.4^{a}
M-5	794.2 ± 1.54^{g}	38.93 ± 0.01^{h}	13.62 ± 0.07^{f}	14.30 ± 0.04^{f}	8.55 ± 0.11^{f}	9.37 ± 0.08^{d}	498.1 ± 14.17^{e}	627.70 ± 12.34^{f}	1126 ± 25.68^{f}	4.35 ± 0.60	1352 ± 39.97^{b}	5858 ± 773.9^{bc}
M-6	773.3 ± 1.67^{f}	37.18 ± 0.06^{g}	12.67 ± 0.03^{e}	13.38 ± 0.06^{e}	8.02 ± 0.06^{cde}	8.31 ± 0.08^{b}	412.00 ± 3.84^{d}	461.8 ± 9.82^{e}	873.8 ± 9.91^{e}	3.82 ± 0.35	1341 ± 15.83^{b}	5111 ± 458.8^{bc}
M-7	767.5^{f} ± 2.14	36.28 ± 0.11^{f}	11.48 ± 0.06^{bc}	11.88 ± 0.13^{bc}	7.44 ± 0.05^{b}	8.11 ± 0.09^{b}	317.9 ± 6.05^{b}	391.40 ± 11.99^{c}	709.2 ± 17.9^{b}	4.00 ± 0.35	1317 ± 39.45^{b}	5247 ± 325.1^{bc}
M-8	655.8 ± 3.52^{b}	32.15 ± 0.06^{c}	10.78^{a} ± 0.05	11.40 ± 0.07^{a}	7.63 ± 0.03^{b}	7.95 ± 0.04^{b}	313.7 ± 2.64^{b}	360.1 ± 5.56^{b}	674.8 ± 8.86^{b}	3.5 ± 0.19	1264 ± 47.22^{ab}	4430 ± 312.7^{abc}
Overall Pooled	716.5 ± 10.59	34.37 ± 0.41	11.85 ± 0.12	12.32 ± 0.14	7.81 ± 0.07	8.26 ± 0.08	367.70 ± 9.71	423.8 ± 13.32	791.40 ± 22.62	3.82 ± 0.12	1271 ± 22.68	4852 ± 166.3

Means bearing different superscript within a column differ significantly ($p < 0.05$).
BWt = Body-weight, SC = Scrotal Circumference, RT.L = Right testicular length, LTL = Left testicular length, RTD = Right testicular diameter, LTD = Left testicular diameter, RTV = Right testicular volume, LTV = Left testicular volume, PTV = Pool testicular volume, EV = Ejaculate volume

Table 2: Correlation coefficients (r= values) among different testicular biometry indices and seminal attributes of Murrah bulls

Particulars	*BWt*	*SC*	*RTL*	*LTL*	*RTD*	*LTD*	*RTV*	*LTV*	*PTV*	*EV*	*Sp. Conc/mL*
SC	0.88**										
RTL	0.67**	0.79**									
LTL	0.71**	0.81**	0.98**								
RTD	0.67**	0.66**	0.70**	0.72**							
LTD	0.75**	0.75**	0.76**	0.78**	0.93**						
RTV	0.70**	0.73**	0.88**	0.89**	0.93**	0.91**					
LTV	0.73**	0.82**	0.90**	0.91**	0.88**	0.95**	0.93**				
PTV	0.73**	0.79**	0.91**	0.92**	0.91**	0.95**	0.98**	0.99**			
EV	0.09	0.25*	0.30*	0.28*	0.21*	0.26*	0.25*	0.32*	0.30*		
Sp. Conc/mL	-0.07	-0.02	0.14	0.11	0.08	0.10	0.13	0.10	0.12	-0.19	
Total Sp./E	0.07	0.25*	0.36*	0.33*	0.27*	0.32*	0.32*	0.38*	0.36*	0.89**	0.27*

*Significant at 5%, ** Significant at 1%
BWt = Body-weight, SC = Scrotal Circumference, RT.L = Right testicular length, LTL = Left testicular length, RTD = Right testicular diameter, LTD = Left testicular diameter, RTV = Right testicular volume, LTV = Left testicular volume, PTV = Pool testicular volume, EV = Ejaculate volume.

(r=0.88). Likewise, Left testicular diameter was positively correlated with right testicular volume (r=0.91) and left testicular volume (r=0.95).

Testicular Volume

The mean testicular volume (cm^3) was observed to be 791.40 ± 22.62, and it varied from 583.00 ± 15.27 to 1126 ± 25.68 cm^3. The testicular volume of right and left testis observed are separately presented in Table 1, which differed significantly due to the presence of difference in the age of experimental bulls. In the present study testicular volume was higher than that recorded by Kumar and Srivastava (2017).

Testicular volume of bulls was significantly ($p < 0.01$) and positively correlated with body-weight (r=0.73), scrotal circumference (r=0.79) and had low correlations ($p < 0.05$) with ejaculate volume (r=0.30), and sperm count/ejaculate (r=0.36) (Table 2). The present findings on correlations were in agreement with the earlier studies on Murrah bulls (Pant *et al.,* 2003; Srivastava, 2011) and bucks (Patel *et al*., 2021).

Physical Attributes of Fresh Semen

Ejaculate Volume

The average ejaculated volume of semen was recorded to be 3.8 ± 0.12 with the range of 3.22 ± 0.14 to 4.35 ± 0.60 mL (Table-01). This was comparable with the reports of Saini *et al*. (2017) and Pathak *et al*. (2018) but was higher than that recorded by Bhakat *et al*. (2015) and lower than the observations of Isnaini *et al*. (2020). The ejaculate volume is influenced by various factors like age (Bhatt *et al.,* 2002), individuality and collection methods, the season of study (Srivastava, 2011), and frequency of semen collections. Variations can also occur due to semen collector/attendant skill and the temperature of the artificial vagina (Younis, 1996). The correlation coefficients of ejaculate volume with various testicular biometry markers and semen concentration presented in Table 2 revealed that semen volume was highly significantly ($p < 0.01$) and positively correlated with total sperm concentration per ejaculate (r=0.89), and had weak correlations ($p < 0.05$) with scrotal circumference (r=0.25), right testicular length (r=0.36), left testicular length (r=0.33), right testicular diameter (r=0.27), left testicular diameter (r=0.32), right testicular volume (r=0.32), left testicular volume (r=0.38), and total testicular volume (r=0.36). The volume of semen showed a negative correlation with sperm concentration per mL (r=–0.19) and corroborated with the finding of Sirvastava (2011) and Chaudhary *et al*. (2017).

Sperm Concentration/mL

The average sperm concentration (million/mL) in Murrah buffalo bulls recorded was 1271 ± 2.68, and it varied significantly ($p < 0.05$) among the bulls with a range of 1075 ± 54.04 to 1412 ± 38.61 (Table 1). The present findings regarding sperm concentration per mL were similar to the observation of Kanchan *et al*. (2010) but higher than that reported by Bhakat *et al*. (2015), Saini *et al*. (2017), Isnaini *et al*. (2020), and lower than the observation of Srivastava (2011), Kumar (2014), Pathak *et al*. (2018). The wide variation in sperm concentration has been attributed to factors like season, individuality, age of bull, sexual excitement, frequency of semen collection, etc. (Tomar, 1986). The sperm concentration/mL was significantly positively correlated with total sperm output/ejaculate (r=0.27).

Sperm Output/Ejaculate

The mean (± SE) sperm output/ejaculate in Murrah bulls varied between 3786 ± 166.40 and 5858 ± 773.90 million with a mean of 4852 ± 166.3 million (Table 1). The sperm count per ejaculate differed significantly among the bulls. Javed *et al*. (2000) reported lower sperm concentration ($p < 0.05$) in older than younger bulls. Moreover, it showed significant positive correlations with most testicular biometric and seminal attributes studied (Table 2). However, Younis (1996) reported a non-significant difference in sperm concentration per ejaculate among the bulls of young, adult, and old age groups. Variation in concentration might be because the concentration of spermatozoa varies with the bull feeding regimen, reproductive health, testicular size, testicular parenchyma, the season of the year, and different geographical localities (Srivastava, 2011).

Conclusion

The study concluded that higher correlations of scrotal circumference with body-weight, testicular markers, and seminal attributes indicate that scrotal circumference is a good indicator to judge the bulls for semen production and predict its quality.

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