RESEARCH ARTICLE

Relationship among testicular, physical and semen quality parameters of Murrah buffalo breeding bulls

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Abstract: One hundred and thirty mature Murrah buffalo bulls under regular semen collection were selected from five different centres located in Haryana and Punjab and 4 ejaculates of each bull were evaluated. The bull's parameters viz. body condition score (BCS), scrotal circumference (SC) and scrotal surface temperature gradient (SSTG) in One hundred and thirty bulls, and rump fat thickness (RFT) and testicular covering thickness (TCT) of 38 bulls was measured. BCS (1-5) of each bull was recorded by visual observation and palpation, SC was measured using measuring tape (cm), TCT and RFT were measured using ultrasonography (mm) and scrotal surface temperature was recorded (^{0}C) using infrared camera. Semen quality attributes viz. ejaculate volume (EV, ml), mass motility (MM, 0-5), sperm concentration (SPC, million/ml), sperm motility (SM, %), viability (%) and sperm abnormality (SA, %) of each ejaculate were recorded with standard procedure. The correlation matrix of these parameters showed that BCS was positively correlated with RFT ($r=0.604$) ($P<0.01$) and SC ($r=0.201$) ($P<0.05$), negatively ($P<0.05$) with SSTG ($r=-0.177$). Scrotal circumference of bulls had significant ($P<0.01$) positive correlation with TCT ($r=0.441$), EV ($r=0.352$), MM ($r=0.293$), SM ($r=0.261$), viability ($r=0.277$) and RFT ($r=0.343$) ($P<0.05$). A positive correlation ($r=0.549$) ($P<0.01$) between TCT and RFT was also obtained. Whereas, SSTG was negatively correlated with SA ($r= -0.169$) and positively with MM ($r=0.298$) ($P<0.01$) and SPC ($r= 0.201$) ($P<0.05$). It can be concluded that the bull's parameters studied were correlated to each other in which scrotal circumference and scrotal surface temperature gradient showed significant positive correlation with semen quality attributes.

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Introduction

India is the largest milk producer in the world, producing about 146.3 Million Tones of milk with 6.3% annual growth rate (DADF, 2015). Buffaloes are about half of the cattle population but contribute > 50% of the total milk production in the country. However, the average milk production of buffalo is still ~4.71 kg per day (DADF, 2014). The productivity of these animals can be improved using superior quality male germplasm of high genetic potential which is actually lacking.

Semen production may depend on physical health status of bulls (Chacon *et al*., 2002). Body condition score (BCS) gives an immediate idea about nutritional status of animals. Scrotal circumference (SC) is a most accurate indicator of semen quality (Pant *et al*., 2003). Testicular size is directly related to the total mass of sperm producing tissues, onset of puberty in bulls and the fertility of female progeny (Ashwood, 2009). It is highly repeatable and its heritability estimate ranges from 0.26 (Meyer *et al*., 1990) to 0.78 (Heyns, 1987) and has high correlation with body weight (Pant *et al*., 2003; Vale *et al*., 2004; Singh *et al*., 2010). In general, as scrotal circumference increases in yearling bulls, the mass motility, percent normal sperm, sperm concentration and total sperm output also increases, while the percentage of sperm abnormalities decreases (Knights e*t al.*, 1984).

Scrotal surface temperature (SST) and testicular covering thickness (TCT) plays an important role to assess the testicular thermoregulation, spermatogenesis and quality semen production in bulls. Infrared thermography is a non-invasive method to determine scrotal surface temperature in bulls (Coulter, 1988). Bulls with scrotal surface temperature gradients between 4-6°C from the top to the bottom had a lower incidence of primary sperm defects compared to bulls with less than 4°C gradients (Coulter, 1988). Therefore the present study was undertaken to find out the relationship among physical, testicular and semen quality parameters of Murrah buffalo breeding bulls.

Material and Methods

Healthy Murrah buffalo breeding bulls (n=130) in regular semen collection were selected for this study from different locations, viz., Artificial Breeding Research Centre of National Dairy Research Institute, Karnal, Haryana; Central Institute for Research on Buffalo, Hisar, Haryana; Frozen Semen Stations (Semen Bank of Haryana Livestock Development Board), Jagadhri and Hisar, Haryana and Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. The maximum ambient temperature in this goes up to 40-46^{0}C during summer and minimum about 1.5-4.0^{0}C during winter and relative humidity varies from 5-100 percent during the year. Bulls were housed in individual pens with the orientation of east-west direction through its long axis. The bulls had free access to fresh drinking water throughout the day. All the Bulls were fed according to standard feeding schedule along with *ad lib* seasonally available green fodder. The bulls were made to exercise, the day prior to semen collection in the rotator bull exerciser. Vaccination, deworming and other herd-health programme were followed as per the standard schedule.

Body condition score

A technique body condition scoring (BCS) has been described to assess the body condition of the bulls by visual observation and palpation of seven skeletal check points (Spinous processes of lumbar vertebrae, transverse processes of lumbar vertebrae, space between 12th and 13th ribs, sacral crest, sacral crest and hooks, hooks and pins, tail head region). Based on the amount of fat reserves the bulls were scored in a 1 to 5 scale using 0.5 increments. A score of 1 indicates emaciated, 2 indicates thin, 3 indicates average, 4 indicates fat and 5 indicates obese condition. During experiments the body condition of bull was scored two times at one week interval on each five bull stations and then the average value were taken for this study.

Scrotal circumference

The scrotal circumference measured by pulling the testicles firmly into the bottom of scrotum by placing the thumb and fingers laterally on the side of neck of the scrotum and pushing ventrally down. A scrotal circumference measuring tape was slipped over the widest portion of scrotum and scrotal circumference was measured in centimetres (Pant *et al.,* 2003).

Scrotal surface temperature

Infrared thermography was used to determine the scrotal surface temperature (°C) of Murrah buffalo bulls. FLIR_i7 infrared camera was used for taking the thermographic images. The mean scrotal surface temperature of each bull was taken by positioning the infrared thermal camera 1 meter away from bull's testis oriented perpendicular to the scrotum (Menegassi *et al.,* 2015). The images were then analysed using FLIR Tools® software to determine the temperature at three different places of scrotum i.e. top, mid and bottom. The temperature from these three places were measured using a line 1-pixel high located from side to side to the scrotal image for each region. The temperature gradient was then determined between top and bottom of scrotum.

Testicular covering thickness and rump fat thickness

The testicular covering thickness and the diameter of rump fat thickness was measured using ultrasonography (KAIXIN KX 2600, Xuzhou Kaixin Electronic Instrument Co. Ltd. China) of 38 bulls (25 bulls at ABRC, Karnal, 7 bulls at CIRB, Hisar and 6 bulls at GADVASU, Ludhiana). The testicular coverings include layers outside to testicular parenchyma i.e. tunica albuginea, tunica vaginalis, fasciae (fatty tissues), dartos and skin. The linear ultrasonographic probe of 6.5 MHz was placed longitudinally on the dorsal surface of testicles. A hyperechoic line, i.e. tunica albugenia, just above the testicular parenchyma was seen. The distance from tunica albugenia to upper most dorsal layer of scrotal skin was measured in mm. The rump fat thickness includes the thick fat layer and the 2 thin subcutaneous fascia layers. The distance between the hook and pin bone was measured by measuring tape and the centre point was marked for placing of ultrasonographic linear probe (Singh *et al*., 2015). The ultrasonographic probe of 6.5 MHz was placed longitudinally and slightly dorsal to the centre point between pin bone and hook bone and the thickness of rump fat was recorded in mm.

Semen quality

The bulls were thoroughly washed, cleaned, and dried at least 20 minutes before semen collection in early morning. Semen was collected twice in a weak at regular interval by using bovine Danish model artificial vagina (IMV model-005417) (42-45°C) over a male dummy bull. Each ejaculate was placed in a water bath at 32°C immediately after collection. Quality of fresh semen was assessed in terms of ejaculate volume (EV, ml), mass motility (MM, 0-5 scale), sperm motility (SM, %), sperm concentration (SPC, IMV photometer), viability and morphological sperm abnormality (SA, eosin-nigrosine staining) by using phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan) equipped with a heating stage (37°C).

Statistical analysis

The relationship among testicular, physical and semen quality parameters was calculated by Pearson's correlation coefficient (r) using SPSS version 20 (statistical analysis software).

Results and Discussion

In this study, a significant ($p<0.01$) positive correlation ($r=0.604$) between BCS and RFT was obtained. BCS also had significant ($P<0.05$) positive correlation ($r=0.201$) with SC and negative correlation with SSTG ($r= -0.177$) ($P<0.05$). Similar trend of association between BCS (average, good, very good and

Table 1: Correlation matrix among physical, testicular and Semen parameters of Murrah buffalo breeding bulls

	BCS	SC	RFT	TCT	SSTG	EV	MM	SPC	SM	Viability	SA
BCS	1										
SC	0.201*	1									
RFT	0.604**	0.343*	1								
TCT	0.318	0.441**	0.549**	1							
SSTG	-0.177*	0.107	-0.153	-0.090	1						
EV	0.062	0.352**	0.215	0.413**	0.005	1					
MM	-0.020	0.293**	0.146	0.158	0.298**	0.267**	1				
SPC	0.009	0.153	-0.289	-0.208	0.201*	-0.121	0.497**	1			
SM	-0.008	0.261**	0.102	0.064	0.159	0.199*	0.874**	0.473**	1		
Viability	0.064	0.227**	0.236	0.161	0.123	0.155	0.788**	0.388**	0.927**	1	
SA	0.097	-0.041	0.196	0.243	-0.169	-0.016	-0.420**	-0.361**	-0.514**	-0.452**	1

n=38 for RFT and TCT; n= 130 for BCS, SC, SSTG, EV,MM, SPC, SM, Viability and SA
BCS= Body Condition Score, SC= Scrotal Circumference, RFT= Rump Fat Thickness, TCT= Testicular Covering Thickness, SSTG= Scrotal Surface Temperature Gradient, EV= Ejaculate Volume, MM= Mass Motility, SPC= Sperm Concentration, SM= Sperm Motility, Viability= Sperm Viability and SA= Sperm Abnormality. (**p<0.01, *p<0.05)

excellent) of breeding bulls and their sperm abnormalities was found (Sarder, 2008). Since, no information was available on these parameters in cattle bulls in general and buffalo bulls in particular, hence, the values obtained in this study could not be compared as such. However, it was an attempt to correlate the BCS, RFT, SC and SSTG in buffalo breeding bulls. In general the rump fat deposition was increased with increased in body health status of the bull. Relationship between back fat thickness (BFT) and total body fat (TBF) content was found highly significant and it showed that a change in BFT of 1 mm equates to approximately 5 kg of TBF content (Schroder and Staufenbiel, 2006). If bull is not exercised regularly its scrotal circumference is increased due to deposition of fatty tissues into the scrotum and scrotal surface temperature gradient is decreased due to improper thermoregulation (fatty tissues work as insulator for heat exchange).

In present study a significant positive relationship of RFT with TCT (r= 0.549) (P<0.01) and SC (r= 0.343) (P<0.05), and negative relationship with SSTG (r= -0.153) was obtained. Testicular covering thickness (TCT) was also found to be positively correlated with SC (r= 0.441) (P<0.01) and negatively with SSTG (r= -0.090).

It shows that TCT and SC increased and SSTG decreased with increase in RFT of bulls. It may be due to deposition of fat together in all body parts including RFT, TCT and SC. In scrotum, as level of fatty tissues increases that affects thermoregulation (insulation effect of fatty tissues) therefore the temperature difference between top and bottom parts of scrotum remains very less. Due to this low temperature gradient between top and bottom of the scrotum the semen quality is affected.

In the present study significant (P<0.01) positive correlations of SC with ejaculate volume (r=0.352), mass motility (r=0.293), sperm motility (r=0.261) and sperm viability (r=0.227) were found. The results obtained are in accordance with the findings of Kumar *et al.* (2015); Pant *et al*. (2003), but contrary to the results in Murrah buffalo bulls (Das and Tomar, 1995 and Ghosh, 2004). SC was favourably correlated to testes mass, sperm production, and semen quality, age at puberty and age at sexual maturity in young bulls (Swanepoel and Heyns, 1990; Brito *et al*., 2007) and favourable genetic relationship with semen quality of bulls (Knights e*t al*., 1984). In general, as scrotum circumference increased in yearling bulls, the mass motility, percent normal sperm, sperm concentration and total sperm output also increased while the sperm abnormality decreased (Knights *et al*., 1984). In this study also, a negative relationship (r= -0.041) of SC with sperm abnormalities was obtained.

The SSTG showed a significant positive correlation with MM (r=0.298) (p<0.01) and sperm concentration (r=0.201) (p<0.05), and a negative correlation with sperm abnormality (r= -0.169) was obtained. Except sperm concentration, scrotal temperature gradient (STG) was not significantly correlated with the various semen quality attributes, such as sperm concentration, % progressive motility, live sperm, and sperm morphology in beef bulls (Lunstra and Coulter, 1993). Whereas, temperature gradient had a significant (P<0.05) positive correlation with sperm motility (r=0.36) and vigour (r=0.35) (Menegassi *et al*., 2015). Finally it was found that all buffalo bull's parameters were related to each other and influenced semen quality.

Conclusions

From the findings, it can be concluded that scrotal circumference and scrotal surface temperature gradient are useful indicator of semen quality and can be added in breeding soundness evaluation of buffalo bulls.

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