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Scrotal infrared thermography and testicular biometry: Indicator of semen quality in Murrah buffalo bulls

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ABSTRACT

This aim of this study was to assess the relationship, if any, of scrotal surface temperature gradient (SSTG), scrotal circumference (SC) and testicular covering thickness (TCT) with semen quality in Murrah buffalo breeding bulls. For this, buffalo breeding bulls (n = 130) were selected from four different semen centres. The ejaculates of each bull were evaluated for ejaculate volume (EV), mass motility (MM); concentration (SPC), motility (SM), viability and abnormalities (SA). The SSTG, SC and TCT of individual bulls were quantified using digital infrared thermography, measuring tape and ultrasonography, respectively. The bulls were divided into three groups on the basis of SSTG (≤ 4 °C, 4.1 to 6.4 °C and ≥ 6.5 °C), and SC (< 31, 31 to 35 and > 35 cm) and into two groups on the basis of TCT (5 to 7.2 and 7.4 to 10.4 mm). Results indicated the bulls with a larger temperature gradient and larger SC produced greater quality semen than those with a lesser temperature gradient. The MM (P < 0.01) and SPC (P < 0.05) varied among the groups along with SSTG. Among the SC groups the EV, MM, SPC and SM (P < 0.01), and viability (P < 0.05) varied as did the SC. The bulls with a lesser TCT had a lesser SA (P < 0.05) as compared to the group with the greater TCT. Buffalo bulls having a greater SSTG, SC and lesser TCT produced semen of greater quality and these variables may be used as criteria for breeding soundness evaluation.

1. Introduction

Artificial Insemination (AI) is the most widely used technique for dissemination of superior genetic material of outstanding males in livestock species. Superior male germplasm identification and its propagation are of utmost importance for breed improvement (Singh and Balhara, 2016). Murrah, one of the dairy buffalo breeds in largest numbers, is used for upgrading local/non-descript buffalo in India and elsewhere (Ahirwar et al., 2018). There are standard selection criteria for breeding dairy and beef bulls but this is lacking for buffalo bulls.

Infrared thermography has been used to quantify scrotal surface temperature (Ahirwar et al., 2017) for assessing scrotal thermoregulation (Coulter et al., 1988), semen quality and fertility in beef bulls (Lunstra and Coulter, 1993) and to evaluate the physiological responses of animals to high temperatures (Knizkova et al., 2007; Paim et al., 2013). This technique is a simple, non-contact and efficient method to determine scrotal surface temperature (SST) in bulls (Coulter et al., 1988). In infrared thermograhic imaging

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of a bull's scrotum having apparently normal testicular thermoregulation, temperature decreases from the dorsal to ventral area of the testis (Kastelic, 2014; Silva et al., 2018). The beef bulls with scrotal surface temperature gradients between 4 and 6 °C from the dorsal to the ventral areas had a lesser incidence of primary sperm defects as compared to the bulls with less than a 4 °C gradient (Coulter et al., 1988).

Testicular size is directly related to the total mass of sperm producing tissues, onset of puberty in *Bos indicus* bulls and the fertility of female progeny (Ashwood, 2009). Testicular size determinations are highly repeatable and the heritability estimates for testicular size ranges from 0.26 to 0.78 in beef bulls (Meyer et al., 1990; Heyns, 1987). In general, scrotal circumference has been correlated with semen quality in bulls (Knights et al., 1984; Pant et al., 2003).

Fatty tissue depositions in the scrotum affects the external covering thickness for the testis. The fat inside the scrotum functions as an insulator that may affects the scrotal/ testicular thermoregulation by reducing the amount of heat that can be radiated from the scrotum, and as a result the testis temperature is greater in beef bulls with a larger amount of external tissue covering and the semen quality is less (Coulter et al., 1997). Such information is lacking for buffalo bulls, hence, the present study was conducted to evaluate the effect of scrotal surface temperature gradient, thickness of testicular covering and testicular size on semen quality of Murrah breeding bulls.

2. Material and methods

Murrah buffalo breeding bulls (n = 130) were selected from four different organization of northern arid and semi-arid region of India (ICAR- National Dairy Research Institute, Karnal, Haryana; ICAR- Central Institute for Research on Buffalo, Hisar, Haryana Frozen Semen Station, Haryana Livestock Development Board, Hisar, Haryana and Frozen Semen Station, Haryana Livestock Development Board, Jagadhri, Haryana. The maximum ambient temperature ranges from 40 to 48 °C during the summer months and the minimum ranges from 1 to 4 °C during the winter months with the relative humidity varying from 45% to -90% during the year.

2.1. Scrotal surface temperature

Infrared thermography was used to determine the scrotal surface temperature (°C) of Murrah buffalo bulls using an infrared camera ((Model: FLIR_i7, Make: FLIR®Systems, Inc.). The mean scrotal surface temperature of each bull was taken 1 day before semen collection by positioning the infrared thermal camera 1 m from the bull's testis with the orientation being perpendicular to the scrotum (Menegassi et al., 2015). Thermographic images of each testis were analysed using software (FLIR Tools®) to determine the temperature at three specific sites on the scrotum (i.e., dorsal, mid and ventral). Along with the testis assessments, the microclimatic temperature (MCT) immediately below the testis of each bull was also recorded. The temperature gradient was then ascertained by determining the difference in temperature values at the dorsal and ventral areas of the scrotum (Fig.1).

2.2. Testicular covering thickness

Testicular covering, which included the five layers from the skin surface to the testicular parenchyma (i.e., skin, dartos muscle, fasciae -fatty tissues, tunica vaginalis, and tunica albuginea). These testicular layers were measured on 38 Murrah bulls using ultrasonography (KAIXIN KX 2600, Xuzhou Kaixin Electronic Instrument Co. Ltd. Jiangsu, China). To quantify the thickness, a linear ultrasonographic probe of 6.5 MHz was placed longitudinally on the dorsal surface of testicle at three different places and the average thickness was calculated (Fig. 2A). A hyperechoic line from the testicular parenchyma to the dorsal layer of the scrotal skin was inserted so that actual the distance from the tunica albugenia to the upper most dorsal layer of the scrotal skin could be determined (Pant et al., 2003; Fig. 2B).

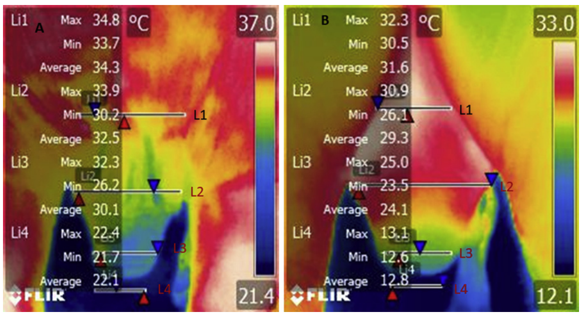

Fig. 1. Scrotal surface temperature (°C) of Murrah buffalo bulls was determined by Infrared camera (Model: FLIR_i7, Make: FLIR®Systems, Inc.) at different microclimatic temperature just below the testis of each bull; A. high microclimatic temperature (22.1 °C) and B. low microclimatic temperature (12.8 °C).

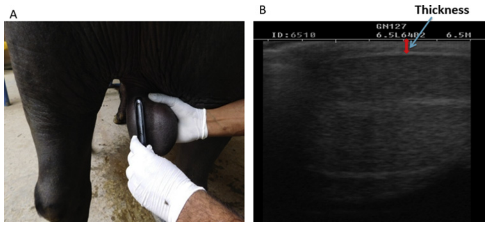

Fig. 2. A. A linear ultrasonographic probe of 6.5 MHz was placed longitudinally on the dorsal surface of testicle to measure testicular covering thickness; B. A hyperechoic line i.e. tunica albugenia (arrow), just above the testicular parenchyma was detected, and the distance from tunica albugenia to upper most dorsal layer of scrotal skin was measured.

2.3. Scrotal circumference

Scrotal circumference (SC) was measured after proper restraining of bull in a confinement crate. A scrotal circumference measuring tape was placed over the widest middle portion of scrotum after proper pressure was placed on the testicles to ensure descent of the testis into the bottom of the scrotum and the SC was measured in centimetres (Pant et al., 2003).

2.4. Semen collection and quality assessment

Two ejaculates were collected twice a week from each bull using a bovine Danish model artificial vagina (IMV model-005417) (42–45 °C) for semen collection after the bulls mounted a dummy male used for purposes of semen collection. Immediately after collection, each ejaculate was placed in a water bath at 32 ± 2 °C for assessment. Each semen sample was assessed for ejaculate volume (mL), mass motility (0–5 scale), sperm motility (%) subjectively using a phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan) equipped with a warm stage (37 °C) at 400 X magnification. In addition, sperm concentration (million/mL) was evaluated with a bovine photometer (IMV, L'Aigla, France), and viability (%) and sperm abnormalities (%) were evaluated using eosin-nigrosin staining and assessment was with a phase contrast microscope. Seminal attributes including total sperm per ejaculate (SPCE), total motile sperm per ejaculate (TMSE) and total live sperm per ejaculate (TLSE) were also calculated.

2.5. Experiment design

In present study, the groupings of animals occurred based on scrotal surface temperature gradient (SSTG), SC and testicular covering thickness (TCT). On the basis of SSTG and SC the bulls (n = 130) were categorised into three groups (I, II and III) and on the basis of TCT, the bulls (n = 38) were assigned to two groups (I and II). The SSTG, SC and TCT groups were I - ≤ 4 °C, II - 4.1–6.4 °C and III - ≥6.5 °C; I- < 31, 31–35 and > 35 cm; I - 5–7.2 and II - 7.4–10.4 mm, respectively.

2.6. Statistical analysis

Data for SSTG, SC and TCT were analysed using a one way ANOVA utilising SPSS statistical software (IBM SPSS Statistics 20) and the means were compared using the Duncan multiple range test. Significance level was set at 95% and 99%. Relationships among the testicular and semen quality parameters were determined using the Pearson's correlation coefficient (r^2) and the SPSS software.

3. Results

3.1. Microclimatic temperature and scrotal surface temperature gradient

The MCT below the testis affected the SSTG between the dorsal and ventral parts of the scrotum. The mean value of SSTG was 3.11 ± 0.10, 4.91 ± 0.09 and 8.03 ± 0.26 °C at corresponding mean values of microclimatic temperature below the testis of 24.62 ± 0.95, 23.44 ± 0.91 and 17.38 ± 1.23 °C, respectively. Results also indicate the value of SSTG increased with the decrease in MCT and that SSTG was the greatest (8.03 ± 0.26 °C) when the MCT was the least (17.38 ± 1.23 °C). There was a negative correlation (P < 0.01) between MCT and SSTG (Table 4).

3.2. Scrotal surface temperature gradient and semen quality

Scrotal surface temperature gradient from the dorsal and ventral parts of the scrotum is indicative of the thermoregulation capacity of the scrotum. The range of SSTG in the three different groups was ≤ 4, 4.1–6.4 and ≥ 6.5 °C in Groups I, II and III, respectively. The value for mass sperm motility (P < 0.01) and sperm concentration (P < 0.05) was greater at ≥ 6.5 and less at ≤ 4 °C of the SSTG but it did not vary between the ≤4 and 4.1–6.4 °C groups. Other seminal variables such as sperm motility and viability did not differ among groups (Table 1). The SSTG was positively correlated with sperm mass motility (r = 0.298) (P < 0.01) and sperm concentration (r = 0.20; P < 0.05), whereas there was a negative association with sperm abnormalities (r = -0.17; Table 4).

Table 1
Effect of scrotal surface temperature gradient (SSTG) on semen quality of Murrah breeding bulls (Mean ± SE).

Variables	Scrotal surface temperature gradient (°C)		
	Group 1 (≤ 4 °C) (n = 48)	Group II (4.1– 6.4 °C) (n = 54)	Group III (≥6.5 °C) (n = 28)
Ejaculate volume (mL)	3.45 ± 0.14	3.72 ± 0.13	3.44 ± 0.13
Mass motility (0–5 scale)	3.40^{A} ± 0.07	3.46^{A} ± 0.07	3.73^{B} ± 0.08
Sperm concentration (million/mL)	1098.05^{a} ± 34.34	1123.91^{a} ± 40.69	1265.64^{b} ± 30.05
Sperm motility (%)	76.62 ± 1.12	76.09 ± 1.13	79.14 ± 0.97
Sperm viability (%)	83.90 ± 0.87	83.60 ± 0.75	85.00 ± 0.92
Sperm abnormality (%)	9.31 ± 0.60	8.92 ± 0.63	7.65 ± 0.48
Total sperm (million)	3763.45 ± 178.72	4122.60 ± 182.58	4327.80 ± 189.82
TMSE (million)	2932.55 ± 154.61	3182.51 ± 154.86	3448.15 ± 170.25
TLSE (million)	3188.15 ± 161.30	3468.74 ± 161.01	3700.90 ± 180.72

Mean values with different superscripts A, B, C ($P < 0.01$) or a, b, c ($P < 0.05$) in row differ; TMSE = total motile sperm per ejaculate, TLSE= total live sperm per ejaculate.
Sperm motility and concentration were determined by using a phase contrast microscope and bovine photometer (IMV, L'Aigla, France), respectively, while sperm viability and abnormalities were determined using eosin-nigrosin staining.

3.3. Testicular covering thickness and semen quality

The range of testicular covering thickness was 5.0–7.0 and 7.4 to 10.4 mm in Groups I and II, respectively. Mean values of ejaculate volume and sperm abnormalities were greater ($P < 0.05$), whereas the mean value for sperm mass motility, concentration and motility did not differ among groups. The percentage of abnormal sperm was less ($P < 0.05$) in the group of bulls which had a lesser TCT. Other seminal attributes such as sperm motility and viability did not differ among TCT groups (Table 2).

3.4. Scrotal circumference and semen quality

The range of SC was < 31, 31–35 and > 35 cm in Groups I, II and III, respectively. Results indicated the mean value for ejaculate volume, mass sperm motility, sperm concentration, and sperm motility varied ($P < 0.01$) among the groups. There was a greater value for semen volume, sperm motility and sperm viability in Group III followed by Groups II and I (Table 3). The data were further subjected to correlation analysis to assess the association of different seminal variables with those for SC There was a positive correlation ($P < 0.01$) of SC with semen volume (r = 0.35), mass sperm motility (r = 0.29), individual sperm motility (r = 0.26), sperm viability (r = 0.28), total sperm (r = 0.39), TMSE (r = 0.40) and TLSE (r = 0.39) (Table 4).

4. Discussion

The results from the present study indicate that when there is a greater temperature of the air around the testis, the surface temperature of the scrotum is also greater and the temperature gradient between the dorsal and ventral part of the scrotum is less. In Braford bulls (cross of Hereford bull and Brahman Cow), there were similar findings as those in the present study that the temperature gradient was greater in the winter (4.0 °C) as compared with the summer (0.9 °C) season (Menegassi et al., 2015). The temperature gradient variation occurs due to the arrangement of the vasculature in the scrotum as it is apparently vascularised from the dorsal pole of the testis to the ventral pole, while the testicular artery traverses dorsally from the ventral to the dorsal part of the testis

Table 2
Effect of Testicular covering thickness on semen quality of Murrah breeding bulls (Mean ± SE).

Variables	Testicular covering thickness (mm)	
	Group I (5.0–7.2 mm) (n = 20)	Group II (7.4–10.4 mm) (n = 18)
Ejaculate volume (mL)	2.75^{a} ± 0.20	3.51^{b} ± 0.26
Mass motility (0-5 scale)	3.10 ± 0.11	3.06 ± 0.14
Sperm concentration (million/mL)	1152.71 ± 69.90	972.95 ± 80.79
Sperm motility (%)	73.72 ± 2.31	71.63 ± 2.94
Sperm viability (%)	81.26 ± 1.81	82.25 ± 1.94
Sperm abnormality (%)	7.72^{a} ± 0.77	11.43^{b} ± 1.28
Total sperm (million)	3100.47 ± 240.67	3394.69 ± 370.66
TMSE (million)	2321.35 ± 190.05	2499.01 ± 308.50
TLSE (million)	2542.60 ± 203.62	2813.28 ± 321.37

Sperm motility and concentration were determined by using phase contras microscope and bovine photometer (IMV, L'Aigla, France) respectively while sperm viability and abnormalities were determined using eosin-nigrosin staining.
Mean values with different superscripts a, b, c ($P < 0.05$), in a row differ; TMSE = total motile sperms per ejaculate, TLSE = total live sperms per ejaculate.

Table 3
Effect of scrotal circumference on semen quality of Murrah breeding bulls (Mean ± SE).

Variables	Scrotal Circumference (cm)		
	Group I (< 31 cm) (n = 16)	Group II (31–35 cm) (n = 62)	Group III (> 35 cm) (n = 52)
Ejaculate volume (mL)	2.97^{A} ± 0.18	3.43^{B} ± 0.13	3.89^{C} ± 0.10
Mass motility (0–5 scale)	3.25^{A} ± 0.15	3.41^{A} ± 0.06	3.67^{B} ± 0.05
Sperm concentration (million/mL)	972.18^{A} ± 67.90	1157.12^{B} ± 34.50	1183.44^{B} ± 30.38
Sperm motility (%)	73.21^{A} ± 2.73	75.91^{AB} ± 1.06	79.33^{B} ± 0.54
Sperm viability (%)	81.57^{a} ± 2.12	83.38^{ab} ± 0.74	85.52^{b} ± 0.48
Sperm abnormality (%)	9.67 ± 1.68	8.80 ± 0.55	8.50 ± 0.35
Total sperm (million)	2878.29^{A} ± 274.69	3894.23^{B} ± 155.69	4556.73^{C} ± 143.34
TMSE (million)	2174.87^{A} ± 241.15	3001.88^{B} ± 135.72	3620.23^{C} ± 119.07
TLSE (million)	2396.71^{A} ± 256.30	3271.21^{B} ± 139.73	3900.12^{C} ± 127.01

Sperm motility and concentration were determined by using phase contras microscope and bovine photometer (IMV, L'Aigla, France), respectively, while sperm viability and abnormalities were determined using eosin-nigrosin staining.
Mean values with different superscripts A, B, C ($P < 0.01$) or a, b, c ($P < 0.05$) in row differ; TMSE = total motile sperms per ejaculate, TLSE = total live sperms per ejaculate.

Table 4
Correlation matrix between climatic temperature, testicular traits and seminal quality of Murrah breeding bulls (n = 130).

	MCT	SSTG	TCT	SC	EV	MM	SPC	SM	Viability	SA	SPCE	TMSE	TLSE
MCT	1												
SSTG	−0.41**	1											
TCT	−0.35*	−0.09	1										
SC	0.03	0.11	0.44**	1									
EV	0.01	0.01	0.41**	0.35**	1								
MM	−0.14	0.29**	0.16	0.29**	0.27**	1							
SPC	0.12	0.20*	−0.21	0.15	−0.12	0.49**	1						
SM	−0.08	0.16	0.06	0.26**	0.19*	0.87**	0.47**	1					
Viability	−0.08	0.12	0.16	0.23**	0.16	0.79**	0.39**	0.93**	1				
SA	0.02	−0.17	0.24	−0.04	−0.02	−0.42**	−0.36**	−0.51**	−0.45**	1			
SPCE	0.09	0.14	0.19	0.39**	0.72**	0.56**	0.57**	0.46**	0.37**	−0.23**	1		
TMSE	0.08	0.16	0.19	0.40**	0.67**	0.67**	0.61**	0.59**	0.51**	−0.28**	0.98**	1	
TLSE	0.08	0.15	0.21	0.39**	0.69**	0.64**	0.59**	0.55**	0.49**	−0.26**	0.99**	0.99**	1

**Indicates correlation is significant at the 0.01 level (2-tailed) and * indicates correlation is significant at the 0.05 level (2-tailed); Sperm motility and concentration were determined by using phase contras microscope and bovine photometer (IMV, L'Aigla, France), respectively, while sperm viability and abnormalities were determined using eosin-nigrosin staining.
MCT = Microclimatic temperature, SSTG = Scrotal surface temperature gradient, TCT = Total covering thickness, SC = Scrotal circumference, EV = Ejaculate volume, MM = Mass motility, SPC = Sperm concentration, SM = Sperm motility, SA = Sperm abnormality, SPCE = Sperm concentration per ejaculate, TMSE = Total motile sperms per ejaculate, TLSE = Total live sperms per ejaculate.

(Kastelic et al., 1995). The temperature of the testes was greater when the testicular location was closer to the arterial supply point and was less at the dorsal testicular pole (Brito et al., 2004). The decreasing trend of scrotal surface temperature from the dorsal pole to the tail of epididymis favoured the maintenance of typical values for seminal variables of buffalo bulls (Silva et al., 2018). The similar results of the present and this previous study also indicate that there was a decreasing trend of temperature from dorsal to the ventral part of the scrotum of buffalo bulls. Kastelic and Brito (2012) and Santos et al. (2014) also observed the factors that reduce the thermal comfort of beef bulls, such as high air temperature and relatively greater relative humidity that also negatively affect the seminal quality of bulls. The increase in SST was associated with a less efficient thermoregulation and increase in air temperature which consequently results in a lesser sperm quality (Berry et al., 2011; Kastelic, 2014).

The results regarding the improvement in sperm mass motility and concentration with increased temperature gradients in the present study are consistent with the findings of Menegassi et al. (2015) because the average mass sperm motility and individual sperm motility was less in the summer (less temperature gradient) compared with other seasons of the year. Similarly Berry et al. (2011) observed that the temperature gradient is related directly to the progressive sperm motility and epididymal sperm reserve, and inversely to ambient temperature (Kastelic et al., 1996). The improvement in mass sperm motility with the increasing SSTG in the present study might be due to the greater sperm concentration and lesser percentage of abnormal sperm in the ejaculate. The lesser sperm concentration when there was a lesser temperature gradient could be due to the increase in testicular temperature. This increase in testicular temperature may have led to an increase in metabolism and testicular oxygen requirement, but testicular blood flow remains relatively constant and this increase does not offset the tissue need for oxygen, resulting in hypoxia and alterations of spermatogenesis (Setchell, 2006). There was no variation in ejaculate volume with the change in temperature gradient of the scrotum. This finding might be associated with the fact that one of the major contributors to ejaculate volume is the accessory sex glands that may not be affected by changes in scrotal temperature (Soleilhavoup et al., 2014). The testicular temperature gradient was less when bulls have an abnormal thermogram, with there being a lesser sperm concentration and value for other seminal variables that are related to fertility (Lunstra and Coulter, 1997; Kastelic and Brito, 2012; Menegassi et al., 2015).

To the best of our knowledge in the present study for the first time there was measurement of the thickness of testicular covering using ultrasonic techniques in buffalo bulls. Scrotal skin thickness was previously assessed using a slide clipper. With the use of this technique, there is a greater than desirable amount of variation in data due to the lack of preciseness in measurements with repeated evaluations on the same animal and consequently inconsistent results. The testicular thickness in buffalo bulls ranges from 5 to10.4 mm. In present study, there was a lesser number of abnormal sperm in the group of bulls which had a lesser thickness of testicular covering than that of those with a greater thickness of testicular covering. It might be due to the larger amount of scrotal heat loss and thermoregulation in the group of bulls which had a lesser thickness of testicular covering. Consistent with these results, Siddiqui et al. (2008) also observed that scrotal skin-fold thickness negatively affected the proportion of sperm with normal heads morphology. The increased thickness of the scrotum covering is associated with deposition of fatty tissues into the scrotum, which functions as insulation and suppresses testicular thermoregulation by reducing the amount of heat that can be radiated from the scrotum (Coulter et al., 1997). It has been well established that for homeostatic testicular function to exist, there should be a 4 to 6 °C lesser testicular temperature than the core body temperature, otherwise, there may be damage to the testicular parenchyma and consequently a reduction in the seminal quality and production of fertile sperm (Garcia et al., 2010; Kastelic, 2014). The thickness of scrotum has an important function in regulating and maintaining the testicular temperature in a thermos-neutral range. If skin thickness of the scrotum is less (thin) with little hair, and large amounts of vasculature, there is a relatively greater radiation and heat loss from the scrotum than when there is a greater thickness to the scrotum, which consequently helps in maintaining a relatively lesser testicular temperature and greater testicular spermatogenesis and sperm viability. It is evident that the scrotal insulation of bulls functions to decrease heat loss which leads to greater testicular temperatures that subsequently affect seminal quality (Barth and Bowman, 1994). Moderate increases in testicular temperature of the bulls submitted to scrotum insulation, markedly reduced sperm production and motility in the ejaculate, and increased the percentage of morphologically abnormal sperm (Kastelic et al., 2001; Fernandes et al., 2008)

In the present study, the SC of 130 adult breeding Murrah buffalo bulls were measured and the mean SC was 37.7 cm (range: 26–44 cm). The relatively larger size of a scrotum is associated with greater seminal quantities due to the greater total mass of sperm producing tissues (Ashwood, 2009). Scrotal circumference is correlated with testes mass, sperm production, and semen quality (Swanepoel and Heyns, 1990; Brito et al., 2007). The findings of a positive correlation of scrotal circumference with ejaculate volume, mass sperm activity and initial sperm motility is consistent with the findings of Kumar et al. (2015) and Pant et al. (2003). Results in the present study are consistent with those of Coulter and Foote (1979) and Knights et al. (1984) where it was reported that the bulls having small sized testicles had a lesser proportion of functional semniferous tubules, lesser sperm output, lesser semen quality, and greater percentage of morphologically abnormal sperm.

In conclusion, the present study was conducted on a large number of buffalo breeding bulls at different semen stations. It is evident that the bulls having a greater scrotal thermoregulation capacity, which was measured as SSTG and TCT, produced semen with greater quality sperm. The bulls with a lesser TCT, greater temperature gradient and SC produced semen of a greater quality than the bulls without these characteristics. The SSTG, TCT, and SC, therefore, can be used as quality indicators of semen production in buffalo bulls.

Author contributions

SKY, AS, PK and SK involved in semen evaluation and cryopreservation. PS, SKY and SVS performed statistical analysis. PS, SKY and PK designed the work and prepared the manuscript.

Declaration of Competing Interest

The authors declare that they do not have any conflict of interest.

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