Study on semen quality in relation to scrotal surface temperature gradient, testicular covering thickness and scrotal circumference in Murrah bulls

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Abstract
Present study was carried out to evaluate the effect of scrotal surface temperature gradient, testicular covering thickness and scrotal circumference on semen quality in Murrah bulls. Murrah buffalo bulls (n = 18), were selected from Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, for the present study. Testicular parameters viz. scrotal surface temperature (SST), scrotal circumference (SC) and testicular covering thickness (TCT) of individual bulls were measured. The testicular surface temperature gradient (SSTG) was also measured. In conclusion scrotal surface temperature gradient, thickness of testicular covering and scrotal circumference significantly affected semen quality in Murrah buffalo bulls.

Keywords: bull, scrotal circumference, scrotal surface temperature, semen quality and testicular covering thickness

Introduction
The artificial insemination (AI) is the most commonly used biotechnology in developed and developing countries, which plays a vital role wider dissemination of elite genetics of livestock. When compared to natural mating or embryo transfer, AI is more successful, economical, and simple in technique (Vishwanath, 2003; Lone, 2018; Mohanty et al., 2018) [21, 15, 18]. The thermoregulation of testes plays a pivotal role in spermatogenesis and production of quality sperm in bulls. For the production of quality sperm, the testicular temperature in bulls must be 2 to 6 °C below body core temperature (Coulter, 1988 and Kastelic et al., 1994) [6]. The testicular temperature should not be elevated above 33–34.5 °C (Barth and Bowman, 1994) [5], and the higher testicular temperature has been found to have adverse effect on sperm production and semen quality in breeding bulls. The elevated temperature leads to dysfunction in sperm production and quality which might be due to impaired function of mitochondria due to altered oxidative metabolism in sperm and production of reactive oxygen species. Besides, ROS are produced during cryopreservation procedures which in turn lead to impaired quality of sperm post-thaw (Lone et al., 2016; Amin et al., 2018) [16, 1]. The optimum temperature temperature of testes for quality sperm production is maintained by countercurrent heat exchange, blood flow, the position of the testes, and sweating (Brito et al., 2004; Gabaldi and Wolf, 2002) [15, 9]. The researchers have tried to evaluate the testicular temperature with the use of sensors that are inserted into gonads of animal; however, they are not free from risks (Coulter, 1988) [6]. Therefore, as an alternative approach using a non-invasive infrared thermography (IRT) method, Coulter (1988) [6] evaluated testicular temperature and reported no differences between
these measurements using the invasive sensors and IRT. The temperature of scrotal surface has been found to be correlated with testicular temperature, and may give detailed information regarding the ability of the bull to regulate the testicular temperature (Coulter et al., 1988) [6]. It is reported that IRT may be an efficient tool for accurate prediction of thermoregulation of testes, besides semen quality and bull fertility (Kastelic et al., 2001) [10]. The information regarding testicular biometry and testicular thickness covering may play an efficient role in understanding thermoregulation of testes and semen quality of bulls. So the present study was designed to evaluate the effect of scrotal surface temperature gradient, testicular covering thickness and scrotal circumference on semen quality in Murrah bulls.

Material and Methods
In the present study, healthy Murrah buffalo bulls (n=18) used in regular semen collection, were selected from Artificial Breeding Research Centre of ICAR-National Dairy Research Institute, Karnal, Haryana. All the selected bulls were maintained under regular semen collection and semen was collected weekly twice from each bull throughout the peak winter season (15/12/2016 to 15/02/2017). The maximum ambient temperature goes up to 40-48 °C during summer and minimum about 1-4 °C during winter and relative humidity varies from 5-97 percent during the year. Bulls were kept in individual pens under loose housing system with shed orientation of east-west direction through its long axis. The bulls had free access to fresh drinking water throughout the day with continuous supply of ad lib drinking water. 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 rotary bull exerciser. Vaccination, deworming and other herd-health programme were followed as per the standard schedule of the farm.

Assessment of scrotal surface temperature
Infrared thermography was used to determine the scrotal surface temperature (°C) of Murrah buffalo bulls. After the general inspection, the infrared thermography images were taken with a hand held digital thermal imaging DarviDTL007 camera, image resolution (384 X 288) and measurement range -20°C to +650 °C. Before using the infrared thermographic camera, it was adjusted to the ambient conditions. The ambient temperature and relative humidity were measured in the shadow, at 1-meter height, with a highly accurate digital thermometer close to the chute in which the bull was placed. The camera was set to the ambient temperature and humidity which were just measured and reference calibration was exercised using the cap of the camera, which was stored at ambient temperature, as a reference. Before taking the image, the scrotum was cleaned from manure and mud with a dry towel. The image of the scrotum was taken at a distance of 1 meter. Infrared thermal images were taken three times (Early morning, afternoon and late evening) of the day during peak winter period for all the three breeds. For each bull at least two to four infrared images were taken, depending on the quality of the images. If images seemed out of focus (because the bull moved), extra images were taken. Later, the out of focus images were excluded from the trial and the average of the measurements of the two in focus images from the bull were considered.

Thermal image analysis
Thermal images that were in focus were analysed, using the Darvi TI analysis software. In each scrotal image, five different points (Proximal pole temperature, mid pole temperature, distal pole temperature, right epididymis and left epididymis) on the scrotal surface were selected for analysis. Using a drawing pad, all temperatures on the different points were measured. In the analysis, scrotal surface temperature gradient was considered as the difference between the two points and calculated as temperature difference between the proximal and distal surface of the scrotum. The measurement areas on scrotum included a scrotal surface temperature of the left and right testis, called average testicle temperature and the average caudal epididymal temperature of the left and right testis, called caudal epididymal temperature.

Assessment of thickness of testicular coverings
The thickness of testicular coverings was measured using ultrasonography (KALXIN KX 2600, Zuzhou Kaixin Electronic Instrument Co. Ltd.) of 18 Murrah buffalo bulls. The testicular thickness included layers outside to testicular parenchyma tunica albuginea, tunica vaginalis fasciae dartos and skin. The linear ultrasonographic probe of 6.5 MHz was placed longitudinally on the dorsal surface of testicle. A hyperechoic line i.e. tunica albuginea just above the testicular parenchyma was seen. The distance from tunica albuginea to upper most dorsal layer of scrotal skin was measured in mm.

Assessment of scrotal circumference
For scrotal circumference measurement the testicles were pulled 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 centimeters.

Semen collection and quality assessment
The bulls were thoroughly washed, cleaned, and dried at least 20 min before semen collection in early morning. Semen was collected twice in a week at regular interval by using bovine Danish model artificial vagina (IMV model-005417) (42-45 °C) as per standard procedure. Each ejaculate was placed in a water bath at 32 ± 2 °C immediately after collection. Quality of fresh semen was assessed in terms of ejaculate volume (mL), mass motility (0-5 scale), non-eosinophilic sperm (%) and sperm abnormalities (%) (eosin-nigrosine staining) by using phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan) equipped with a heating stage (37 °C). The mass activity was determined by assessing the motility of the fresh ejaculated sperm. For assessment of this, a drop of fresh semen was placed on a clean, grease free glass slide without cover slip maintained at 37 °C under low power (10×) of microscope (Nikon Eclipse E200, Japan). It was graded on the scale ranging from 0 to +5; where 0 means all dead sperm and +5 indicate semen samples with 100% motile sperm with extremely rapid waves (Lone et al., 2018) [15]. Eosin-nigrosin stain was used to determine non-eosinophilic sperm count and the percentage of sperm abnormalities. The detailed preparation of the eosin-nigrosin stain has been mentioned elsewhere (Balamurugan et al., 2018) [3]. The mixture was thoroughly shaken and filtered through Whatman filter paper. One drop of semen sample was mixed with three drops of stain and the semen-stain mixture was allowed to rest for about 1 min. After 1 min, a thin smear was prepared on a clean, grease free slide, air dried and then observed at 1000× magnification of phase contrast microscope. The sperm which
appeared colourless or white were considered as live and those appeared partially or completely pink coloured were considered as dead. A total of 200 sperm were counted in each slide and percentage of live sperm and percentage of various sperm abnormalities was determined.

Statistical analysis
The effect scrotal surface temperature gradient (SSTG) on semen quality among Groups I, II, and III was analyzed by one way ANOVA using SPSS version 16 and the means were compared by Duncan test. However the effect of thickness of testicular and scrotal circumference on semen quality was analysed by t-test. The P value (<0.05) was considered statistically significant.

Results
Effect of scrotal surface temperature gradient on semen quality
Scrotal surface temperature gradient (SSTG) is the reflection of thermoregulation from top to bottom of the testes. The effect of SSTG on semen quality in buffalo bulls has been depicted in Table 1. The Mean ± SE of scrotal surface temperature gradient (SSTG) was 3.98 ± 0.22 °C and 9.50 ± 0.18 °C, respectively in Group I, II and Group III, respectively. The results revealed that mass activity and non-eosinophilic sperm were significantly (p<0.05) increased in Group II as compared to Group I, which indicates that the percentage of sperm abnormalities increased with increase in the thickness of testicular covering. However, no significant effect of SSTG on mass motility and non-eosinophilic sperm percentage was observed.

Table 1: Effect of scrotal surface temperature gradient on semen quality of murrah bulls (Mean ± SE, n = 108)

| Parameters                  | Group I          | Group II         | Group III         |
|-----------------------------|------------------|------------------|-------------------|
| Volume (mL)                 | 1.50 ± 0.34      | 2.80 ± 0.75      | 2.28 ± 0.54       |
| Mass Activity (0-5 Scale)   | 1.88 ± 0.16      | 2.45 ± 0.03      | 2.74 ± 0.06       |
| Non eosinophilic sperm (%)  | 67.30 ± 1.91     | 76.27 ± 1.27     | 83.02 ± 0.70      |
| Head abnormalities (%)      | 3.20 ± 0.93      | 1.83 ± 0.22      | 0.96 ± 0.24       |
| Midpiece abnormalities (%)  | 3.73 ± 0.56      | 2.37 ± 0.20      | 1.71 ± 0.25       |
| Tail abnormalities (%)      | 4.13 ± 0.53      | 1.67 ± 0.19      | 1.25 ± 0.19       |
| Total abnormalities (%)     | 11.10 ± 1.73     | 5.90 ± 0.17      | 3.92 ± 0.32       |

Group I: Scrotal surface temperature gradient (SSTG) = 3.98 ± 0.22;
Group II: SSTG = 6.68 ± 0.58, and Group II: SSTG = 9.50 ± 0.18. Means bearing different superscripts in upper case letters (A, B, C) differ significantly (P<0.05)

Effect of thickness of testicular covering on semen quality
The Mean ± SE of thickness of testicular covering (TTC) was 4.98 ± 0.91 mm and 6.04 ± 0.20 mm in Group I and Group II, respectively in Murrah bulls. The effect of thickness of testicular covering on semen quality in buffalo bulls has been depicted in Table 2. The percentage of head, midpiece, tail, and total abnormalities were significantly (P<0.05) higher in Group II as compared to Group I, which indicates that the percentage of sperm abnormalities increased with increase in the thickness of testicular covering. However, no significant effect of TTC on mass motility and non-eosinophilic sperm percentage was observed.

Table 2: Effect of thickness of testicular covering on semen quality of murrah bulls (Mean ± SE, n = 108)

| Parameters                  | Group I          | Group II         |
|-----------------------------|------------------|------------------|
| Mass Activity (0-5 Scale)   | 2.46 ± 0.06      | 2.38 ± 0.13      |
| Non eosinophilic sperm (%)  | 76.69 ± 2.81     | 76.87 ± 2.12     |
| Head abnormalities (%)      | 1.00 ± 0.22      | 2.65 ± 0.55      |
| Midpiece abnormalities (%)  | 1.76 ± 0.23      | 3.15 ± 0.39      |
| Tail abnormalities (%)      | 1.33 ± 0.19      | 3.00 ± 0.53      |
| Total abnormalities (%)     | 4.09 ± 0.33      | 8.83 ± 1.28      |

Group I: Thickness of testicular covering (TTC) = 4.98 ± 0.91;
Group II: TTC = 6.04 ± 0.20. Means bearing different superscripts in upper case letters (A, B, C) in row differ significantly (P <0.05)

Effect of scrotal circumference on semen quality
The Mean ± SE of scrotal circumference (cm) were 30.14 ± 0.04 and 33.18 ± 0.38 in Group I and Group II, respectively. The effect of scrotal circumference on semen quality has been presented in Table 3. The results revealed that ejaculate volume and non-eosinophilic sperm percentage were significantly (P<0.05) higher in Group II compared to Group I. The percentage of mass motility, head, midpiece, tail, and total abnormalities did not differ significantly (P>0.05) between Group I and Group II, which indicates that scrotal circumference did not affect these seminal parameters.

Table 3: Effect of scrotal circumference on semen quality of murrah bulls (Mean ± SE, n = 108)

| Parameters                  | Group I          | Group II         |
|-----------------------------|------------------|------------------|
| Volume (mL)                 | 1.70 ± 0.07      | 3.21 ± 0.30      |
| Mass Activity (0-5 Scale)   | 2.02 ± 0.15      | 2.37 ± 0.21      |
| Non eosinophilic sperm (%)  | 72.56 ± 1.84     | 83.40 ± 0.67     |
| Head abnormalities (%)      | 1.79 ± 0.36      | 1.85 ± 0.54      |
| Midpiece abnormalities (%)  | 2.12 ± 0.40      | 2.67 ± 0.37      |
| Tail abnormalities (%)      | 1.90 ± 0.67      | 2.13 ± 0.58      |
| Total abnormalities (%)     | 5.83 ± 0.95      | 6.86 ± 1.30      |

Group I: Scrotal circumference (SC) = 4.98 ± 0.91;
Group II: SC = 6.04 ± 0.20 mm. Means bearing different superscripts in upper case letters (A, B, C) in row differ significantly (P <0.05)

Discussion
In the present study Murrah bulls showed higher scrotal surface temperature gradient produced better quality semen. The results were in consonance with the findings of Yadav (2016) [22] who reported significant improvement of mass motility with increase in scrotal surface temperature gradient (SSTG) as well as decrease in the percentage of abnormal sperm with increase in SSTG. It is also evident in the literature that scrotal surface temperature gradient was more in winter (4.0 °C) as compared to summer (0.9 °C) season (Menegassi et al., 2015) [17]. The difference in the temperature from the dorsal pole of the testis to the ventral pole, which is creating temperature gradient, may be due to arrangement of the vasculature; while the testicular artery ramifies dorsally from the bottom of the testis to the top (Kastelic et al., 1995) [11]. Similar finding were reported in our study, and we found that there was decreasing trend of temperature from top to bottom of each testis. The improvement of semen quality traits with increase in scrotal surface temperature gradient may be associated with effective thermoregulation mechanism of testis and further with spermatogenesis. Lower temperature gradient could be due to rise in testicular temperature which leads to increase metabolism and testicular oxygen demand and resulted in alterations of spermatogenesis (Setchell, 2006) [20]. No significant change in ejaculate volume with change in temperature gradient of scrotum might be associated with the fact that one of the major contributors of ejaculate volume is accessory sex gland that may not get influenced by change in scrotal temperature.

The lower percentage of the abnormal sperm was found in the group of bulls, which had lower thickness of testicular covering (TTC) than the group which had higher TTC, which may be due to the better scrotal heat loss and thermoregulation in the group of bulls which had lower TTC than the other group which had higher TTC. The increased thickness of scrotum is associated with the deposition of fatty tissues into the scrotum, which works as an insulation and hampers the thermoregulation process by increasing the testicular temperature. Earlier reports have revealed that for better testicular functions, the testicular temperature should be 2-6 °C lower than body temperature. The scrotal skin thickness plays an important role in regulating and maintaining the testicular temperature at a desired level. If skin thickness of the scrotum is less (thin) with little hair, and much vasculature, that allows for radiation and heat loss from the scrotum, which consequently helps in maintaining the testicular temperature lower for better functioning of testis. It is evident that bull’s scrotal insulation decreases heat loss leading to increase in testicular temperature, which in turn impairs semen quality (Barth and Bowman, 1994) [4]. The reports have also revealed that testicular insulation in bulls significantly alters the morphology of sperm (Fernandes et al., 2008) [8].

Scrotal circumference is an important testicular parameter, easy to measure and most accurate indicator of semen quality (Pant et al., 2003) [19], testicular size and directly related to the total mass of sperm producing tissues and onset of puberty in bulls (Ashwood, 2009) [2]. The consistent increase scrotal circumference resulted in improvement in the seminal attributes might be due to the increase in the total mass of sperm producing tissues and number of secretory tissues. The positive relationship of the scrotal circumference (SC) with volume of ejaculate and percentage of live sperm was in line with the observations of Pant et al. (2003) [19], who reported positive relationship of SC with ejaculate volume and live sperm. In similar line Kumar et al. (2015) [13] reported significant positive correlation of SC with ejaculate volume, and Knights et al. (1984) [12] also reported that with increase in the size of SC, ejaculate volume increased. The results were further supported by the finding of Coulter and Foote (1979) [1], who reported that the bulls having small sized testicles had decreased proportion of functional seminiferous tubules, reduced sperm output and poor semen quality and elevated percentage of morphologically abnormal sperm.

**Conclusion**

The bulls showed higher scrotal surface temperature gradient, lower thickness of testicular coverings and higher scrotal circumference produced better quality semen. Therefore, scrotal surface temperature gradient, scrotal circumference and thickness of testicular coverings may be used as indicator of quality semen production in Murrah bulls during breeding soundness evaluation. All the traits are equally important from selection point of view.

**Conflict of interest**

Authors have no any conflict of interest.

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**References**

1. Amin BY, Prasad JK, Ghosh SK et al. Effect of various levels of dissolved oxygen on reactive oxygen species and cryocapitation-like changes in bull sperm. Reproduction in Domestic Animals. 2018, 1-8.
2. Ashwood A. Bull reproductive soundness. Brahman news. 2009; 164:1-6.
3. Balamurugan B, Ghosh SK, Lone SA, Prasad JK, Das GK, Katiyar R et al. Partial deoxygenation of extender improves sperm quality, reduces lipid peroxidation and reactive oxygen species during cryopreservation of buffalo (Bubalus bubalis) semen. Animal Reproduction Science. 2018; 189:60-68.
4. Barth AD, Bowman PA. The sequential appearance of sperm abnormalities after scrotal insulation of dexamethasone treatment of bulls. Can Vet J. 1994; 34:93-102.
5. Brito LFC, Silva AED, Barbosa RT, Kastelic JP. Testicular thermoregulation in Bos indicus, crossbred and Bos taurus bulls: relationship with scrotal, testicular vascular cone and testicular morphology, and effects on semen quality and sperm production. Theriogenology. 2004; 61:511-528.
6. Coulter GH. Thermography of bull testes, 12th Technical Conference of Artificial Insemination and Reproduction. Columbia, SC: National Association of Animal Breeders. 1988, 58-63.
7. Coulter GH, Foote RH. Bovine testicular measurements as indicators of reproductive performance and their relationship to reproductive performance and their relationship to productive traits in cattle: A review. Theriogenology. 1979; 11:297-311.
8. Fernandes CE, Dode MAN, Pereira D, Silva AEDF. Effects of scrotal insulation in Nellore bulls (*Bos taurus indicus*) on seminal quality and its relationship with *in vitro* fertilizing ability. Theriogenology. 2008; 70:1560-1568.

9. Gabaldi SH, Wolf A. A importância da termorregulação testicular na qualidade do sêmen em touros. Ciências Agrárias. FEA, adrenalina. 2002; 2:66-70.

10. Kastelic JP, Cook RB, Pierson RA, Coulter GH. Relationships among scrotal and testicular characteristics, sperm production and seminal quality in 129 beef bulls. Canadian Journal of Veterinary Research, Ottawa. 2001; 65:111-115.

11. Kastelic JP, Coulter GH, Cook R. Scrotal surface, subcutaneous, intra testicular, and intra-epididymal temperatures in bulls. Theriogenology. 1995; 44:147-152.

12. Knights SA, Baker RL, Gianola D, Gibb JB. Estimates of heritabilities and of genetic and phenotypic correlations among growth and reproductive traits in yearling Angus bulls. Journal of Animal Science. 1984; 58:887-893.

13. Kumar BSB, Pandita SP, Mallick SR, Mohanty TK, Mandal DK, Mili B. Luteinizing hormone, testosterone and total estrogens response to exogenous GnRH in cross bred bulls with differing semen quality. Livestock Science. 2015; 174:150-153.

14. Lone SA, Prasad JK, Ghosh SK, Das GK, Balamurugan B, Verma MR. Study on correlation of sperm quality parameters with antioxidant and oxidant status of buffalo bull semen during various stages of cryopreservation. Andrologia. 2018, e12970.

15. Lone SA. Possible mechanisms of cholesterol-loaded cyclodextrin action on sperm during cryopreservation. Animal Reproduction Science. 2018; 192:1-5.

16. Lone SA, Prasad JK, Ghosh SK, Das GK, Kumar N et al. Effect of cholesterol loaded cyclodextrin (CLC) on lipid peroxidation and reactive oxygen species levels during cryopreservation of buffalo (*Bubalus bubalis*) spermatozoa. Asian Pacific Journal of Reproduction. 2016; 5:476-480.

17. Menegassi SRO, Barcellos JOJ, Dias EA, Koetz C et al. Scrotal infrared digital thermography as a predictor of seasonal effects on sperm traits in Braford bulls. Int. J Biometeorol. 2015; 59:357-364.

18. Mohanty TK, Lone SA, Kumaresan A, Bhakat M, Kumar R, Baithalu RK et al. Sperm dosage and site of insemination in relation to fertility in bovines. Asian Pacific Journal Reproduction. 2018; 7:1-5.

19. Pant HC, Sharma RK, Patel SH, Shukla HR, Mittal AK, Kasiraj R. Testicular development and its relationship to semen production in Murrah buffalo bulls. Theriogenology. 2003; 60:27-34.

20. Setchell BP. The effects of heat on the testes of mammals. Animal Reprod. 2006; 3:81-91.

21. Vishwanath R. AI: the state of the art. Theriogenology. 2003; 59:571-84.

22. Yadav SK. Relationship of scrotal surface temperature and testicular biometry with semen quality of Murrah buffalo breeding bulls of North Arid to Semi-Arid Region. M.Sc. Thesis, NDRI, Karnal, Haryana, India, 2016.