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Dr. Manish Kumar Kushwaha
Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, India

Mukesh Bhakat
Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, India

TK Mohanty
Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, India

SA Lone
Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, India

R Kumar
Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, India

R Sinha
Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, India

Shashank CG
Animal Physiology Division, ICAR-National Dairy Research Institute, Karnal, Haryana, India

A Rahim
Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, India

Z Danish
Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, India

AR Paray
Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, India

HP Yadav
Artificial Breeding Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana, India

AK Gupta
Animal Genetics and Breeding (AGB), ICAR- National Dairy Research Institute, Karnal, Haryana, India

Correspondence
Dr. Manish Kumar Kushwaha
Ph. D Scholar, Livestock Production and Management, ICAR- National Dairy Research Institute, Karnal, Haryana, India

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

Manish Kumar Kushwaha, Mukesh Bhakat, TK Mohanty, SA Lone, R Kumar, R Sinha, Shashank CG, A Rahim, Z Danish, AR Paray, HP Yadav and AK Gupta

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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. Temperature of scrotum (Proximal Pole Temperature, Mid Pole Temperature, Distal Pole Temperature, Right Epididymis and Left Epididymis) was measured three times a day per week during peak winter season. Semen quality attributes *viz*., volume, mass motility, non-eosinophilic sperm and sperm abnormalities of each ejaculate were evaluated. The TCT and SC were measured by using ultrasonography and measuring tape, respectively. The bulls were divided into three groups on the basis of scrotal surface temperature Gradient (SSTG) (Group I- ≤ 4 ºC, Group II- 4.1- 6.8 ºC and Group III- ≥6.9 ºC) and into two Groups on the basis of TCT (Group I- 4.53-5.28 and Group II- 5.36-7.36 mm) and SC (Group I- 28-31 and Group II- 32-35 cm). Mass activity and non-eosinophilic sperm count was improved significantly (P< 0.05) and head, midpiece, tail and total abnormalities were reduced significantly (P< 0.05) with increase in SSTG. The significant (P< 0.05) effect of TTC on sperm abnormalities was observed. Ejaculate volume and non-eosinophilic sperm count increased significantly (P< 0.05) with increase in SC. In conclusion scrotal surface temperature gradient, thickness of testicular coverings 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 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) [4], 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 -20ºC to +650 ºC. 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, Xuzhou 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 ultrasonograpic 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 abmormalaites. 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 testis. 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, 6.68 ± 0.58 °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 III as compared to Groups I & II, which indicates that higher the SSTG, higher the mass motility and non-eosinophilic sperm percentage. No significant improvement in case of semen volume was observed with change in SSTG. The percentage of head, midpiece, tail, and total abnormalities were significantly (P<0.05) increased in Groups II & III as compared to Group I.

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.

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 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^{A} ± 0.16	2.45^{B} ± 0.03	2.74^{C} ± 0.06
Non eosinophilic sperm (%)	67.30^{A} ± 1.91	76.27^{B} ± 1.27	83.02^{C} ± 0.70
Head abnormalities (%)	3.20^{A} ± 0.93	1.83^{AB} ± 0.22	0.96^{B} ± 0.24
Midpiece abnormalities (%)	3.73^{A} ± 0.56	2.37^{B} ± 0.20	1.71^{B} ± 0.25
Tail abnormalities (%)	4.13^{A} ± 0.53	1.67^{B} ± 0.19	1.25^{B} ± 0.19
Total abnormalities (%)	11.10^{A} ± 1.73	5.90^{B} ± 0.17	3.92^{B} ± 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)			

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^{A} ± 0.22	2.65^{B} ± 0.55
Midpiece abnormalities (%)	1.76^{A} ± 0.23	3.15^{B} ± 0.39
Tail abnormalities (%)	1.33^{A} ± 0.19	3.00^{B} ± 0.53
Total abnormalities (%)	4.09^{A} ± 0.33	8.83^{B} ± 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)		

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^{A} ± 0.07	3.21^{B} ± 0.30
Mass Activity (0-5 Scale)	2.02 ± 0.15	2.37 ± 0.21
Non eosinophilic sperm (%)	72.56^{A} ± 1.84	83.40^{B} ± 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 secretary 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) [7], who reported that the bulls having small sized testicles had decreased proportion of functional somniferous 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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