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Thermal imaging to measure scrotal surface temperature and its use as a tool for breeding soundness evaluation of Zebu bulls

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ABSTRACT

The present study was planned to understand the effect of scrotal surface temperature on semen quality and Infrared thermography as a tool for breeding soundness evaluation of Zebu bulls. The thermal imaging of the scrotum revealed significant differences in temperatures from the neck to the ventral portion of the scrotum. The average neck and ventral temperature of the scrotum was 34.2±0.62°C and 31.22±0.53°C, respectively. The average gradient temperature of the scrotal surface (neck-ventral) was 2.95±0.30°C with a range of 1.2–5.7°C. The MA of bull semen (3.12±0.04 vs. 1.89±0.13), live sperm (73.82±0.88% vs. 59.42±1.58%), HOST (66.67±1.52% vs. 55.49±1.14%) and sperm concentration (1226.36±18.80 vs. 942.19±23.47m/ml) were significantly more in good semen quality bulls than poor semen quality bulls. Total sperm abnormality was significantly lesser in bulls having a larger scrotal temperature gradient. The scrotal skin thickness was significantly more in poor semen quality bulls than good semen quality bulls (5.70±0.12 vs. 5.07±0.13). The scrotal skin thickness was found highly correlated (r-0.77) with the scrotal temperature gradient. So it can be concluded that scrotal surface temperature is related to the semen quality of bulls, and thus the thermal imaging can be used for breeding soundness evaluation of Zebu bulls.

Keywords: Infrared thermography, Scrotal skin thickness, Scrotal surface temperature gradient, Semen quality

In a tropical country like India, summer days are so hot that bulls are subjected to severe heat stress, so the reproductive organs also get affected, which in turn interfere with reproductive processes and ultimately their fertility (Berry *et al.* 2011). Scrotal anatomy, like higher scrotal skin thickness, may aggravate thermoregulation of testis. The testicular temperature may increase on hot days and may impair the spermatogenic cycle and the semen quality, which may result in poor fertility. The high temperatures act on spermatic cells and interfere with the oxidative metabolism of glucose due to mitochondrial dysfunctions and the generation of reactive oxygen species (ROS) (Nichi *et al.* 2006). The thermal stress alters metabolic processes, and the cellular energy reserves get exhausted, leading to new adaptive strategies in homoeothermic metabolism (Baumgard and Rhoads 2013). To maintain metabolic homeostasis for sperm production, the structure of the testis uses such adaptation strategies. Hence for studying bovine physiology and reproductive health, the effects of environmental factors on thermoregulation and reproductive changes are necessary. Ruediger *et al.* (2016) has recommended thermography as a supplementary examination for the reproductive evaluation of bulls. Ahirwar *et al.* (2017) also used infrared thermography to measure scrotal surface temperature (Fig.1). Kushwaha *et al.* (2019) reported that bulls with higher scrotal surface temperature gradient had high-quality semen. Barca Junior *et al.* (2020) found that monitoring the scrotal temperature by infrared thermography dynamics was effective. Scrotal sweat glands provide evaporative cooling, which may get affected by fat deposition. This technique can become a different approach in evaluating bull breeding soundness and predicting fertility (Menegassi *et al.* 2015). Yadav *et al*. (2019) found that scrotal surface temperature gradient was a better indicator of quality semen production in buffalo bulls. So the present study was done to evaluate the effect of temperature gradient on semen quality and to know the importance of thermal imaging for bull breeding soundness evaluation.

MATERIALS AND METHODS

The present investigation was conducted on 18 Sahiwal bulls maintained at Artificial Breeding Research Centre, National Dairy Research Institute, Karnal, Haryana. The bulls were reared under a loose housing system. The floor

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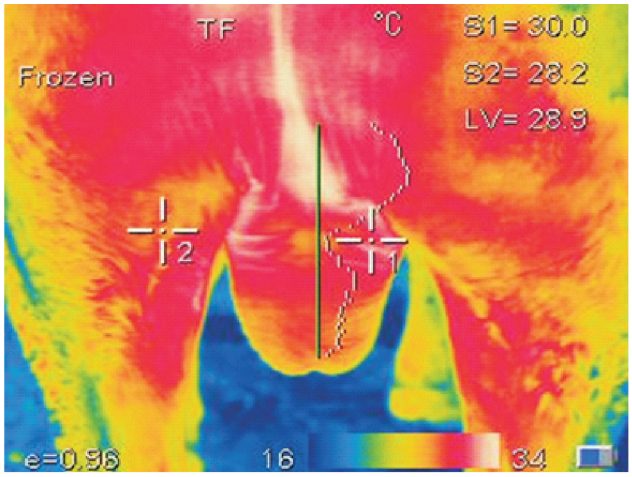

Fig.1. Thermography of scrotum.

of individual pens (30×10 feet) for bulls was concrete, and the roof of the shed had corrugated asbestos. The pens were separated by solid partitions that restricted both direct physical and visual contact of bulls in adjacent pens. The concentrate was provided 2.5 to 3 kg per bull during morning as per ICAR 2013 feeding standard for breeding bulls. *Ad lib*. green fodder was supplied throughout the experimental period. Availability of water was *ad lib.* throughout the day. For the proper healthcare of animals, farm schedule was strictly adapted like vaccination, deworming. One day before semen collection, bulls were exercised. The artificial vagina technique was adapted for semen collection during early morning hours.

The surface temperature was measured at three different places top, mid and bottom of the scrotum of bull (line measurement) with an Infrared thermal camera (FLIR_i7) and the difference between the top (neck) and bottom (epididymis) of scrotal surface temperature was calculated. The rectal temperature of bulls was recorded with a thermometer and animals showing abnormal temperature were excluded from recording the data. The recordings were done in October month during the morning hours when the maximum temperature remains around 25°C. The bull was kept standing for thermographs (images) from behind, after adjusting the camera settings and the distance between the camera and the animal. The stored images were entered into the computer, and with the help of software, the thermographs were analysed. Testicular skin thickness was measured with digital Vernier calipers and ultrasonography. The semen was collected by artificial vagina technique during morning hours. The animals were grouped into good and poor semen quality bulls on the basis of freezability semen. Data were analysed by the DMRT method using SPSS software (Version 16) to assess the effect of temperature gradient on semen quality. The Institution Animal Ethical Committee (IAEC) approved all the procedures of animal experimentation.

RESULTS AND DISCUSSION

The surface temperature was measured at three different places top, mid and bottom of the scrotum of bull (line measurement) with an Infrared thermal camera (FLIR_i7) and temperature gradient was determined between the top and bottom line. The average neck, mid and ventral temperature of the scrotum was 34.2±0.62°C, 32.71±0.63°C, and 31.22±0.53°C. The average gradient temperature of the scrotal surface (neck-ventral) was about 2.95±0.30°C with a range of 1.2–5.7°C.

The thermal imaging of the scrotum revealed significant differences in temperatures from the neck to the ventral portion of the scrotum. The average gradient temperature of the scrotal surface (neck-ventral) was about 2.95±0.30°C with a range of 1.7–5.7°C.

In a similar line, Kushwaha *et al*. (2019) reported that gradient temperatures of bull testis range from 4° to 6.9°C. Scrotum has a positive temperature gradient warmer at the top than the bottom, and our results are similar to the earlier finding in the indigenous bull.

Relationship of scrotal surface temperature gradient and semen quality: The scrotal temperature gradient of good semen quality bulls was more but non-significant than poor semen quality bulls. The semen ejaculate volume was non-significant between the two groups. The MA, live sperm per cent HOST per cent and sperm concentration (million/ml) were significantly (P<0.01) more in good semen quality bulls than poor semen quality bulls. The intact sperm per cent was more non-significant in good semen quality bulls than in poor semen quality bulls (Table 1). Total sperm abnormality (8.70±0.59% vs. 6.09±1.22%) was significantly more (P<0.05) in bulls having lesser scrotal surface gradient temperature.

Brito *et al.* (2004) found that increased testicular volume was associated with semen quality improvement and decreased scrotal surface and testicular temperatures, which aligns with our findings. Similar to our findings, Kastelic *et al.* (2018) found that higher testis temperature adversely affects semen motility percentage, sperm survival, and sperm morphology. Menegassi *et al*. (2015) found that the temperature gradient better was sperm mass motility and concentration, similar to our findings. Abdelhamid *et al*.

Table 1. Scrotal temperature gradient and semen quality of good and poor semen producing Sahiwal bull (Means ± SE, n=18)

Parameter	Good semen quality bulls (9)	Poor semen quality bulls (9)
Temperature gradient (°C)	3.51±0.57	2.36±0.21
Volume (ml)	5.08±0.38	4.27±0.39
Mass activity (1–5)	3.12*±0.04	1.89*±0.13
Live sperm (%)	73.82*±0.88	59.42*±1.58
HOST (%)	66.67*±1.52	55.49*±1.14
Intact acrosome (%)	93.72±0.36	92.52±0.46
Total sperm abnormality (%)	7.14*±0.39	9.50*±0.58
Sperm concentration (10^6/ml)	1226.36*±18.80	942.19*±23.47

*, P<0.05.

(2019) found that an increase in testicular and epididymal temperature results in abnormal sperm morphology, which may be due to an impairment of spermiogenesis and meiosis in men, bulls and rams. Yadav *et al.* (2019) found that bulls having a more significant temperature gradient produced semen of better quality. The thermoregulation of testes depends on the interaction between opposing testicular subtunic and scrotal temperatures gradients in bulls, thus indicating the importance of scrotal surface anatomy in thermoregulation of testicular temperature, which in turn affects sperm survival and quality.

Relationship of scrotal thickness and scrotal surface temperature gradient with semen quality: The average scrotal skin thickness was 5.39±0.11 and the temperature gradient was 3.02±0.33°C. The scrotal skin thickness (5.07±0.13 mm) of good semen quality bulls was significantly (P<0.05) lesser than scrotal skin thickness (5.70±0.12 mm) of poor semen quality bulls. The scrotal skin thickness was found highly (r-0.78) correlated with the scrotal temperature gradient.

The higher fat deposition increases the skin thickness of the scrotum and acts as heat insulation. The more the thickness of the scrotal skin, the more it will impair heat transfer and affect the semen of bulls. The lesser the gradient more it will decrease the semen quality. The scrotal skinfold thickness (SSFT) of Cholistani bulls was found to be 1.10±0.15 cm; the range was 0.98 to 1.32 cm (Mahmood *et al.* 2014). The SSFT has been reported to directly influence semen quality, as the morphology of spermatozoa (Siddiqui *et al.* 2008). The increase in temperature of testis results in increased metabolism and demand of oxygen supply by testis; however, the blood flow of testis does not get disturbed, ultimately resulting in hypoxia and impairment of spermatogenesis (Setchell 2006). The testicular thermoregulatory capability is influenced by testes' morphology, which affects semen quality and sperm production in bulls (Brito *et al.* 2004). Silva *et al*. (2018) found that the temperature gradient from the dorsal pole to the tail of epididymis favoured the maintenance of semen quality in buffalo bulls. Brito (2006) found that bulls fed with more nutritious diets had lesser temperature gradients than bulls fed with less nutritious diets due to increased scrotal skin thickness by fat deposition. Lesser testicular covering thickness in buffalo bulls was producing good quality semen than bulls with thicker testicular covering (Yadav *et al.* 2019).

The scrotal skin thickness is negatively correlated with the scrotal temperature gradient, while scrotal temperature gradient is positively related to semen quality. Kushwaha *et al.* (2019) reported that the temperature gradient of the scrotal surface was a better indicator of semen quality than the testicular covering thickness of bulls. Thus, it can be concluded that thermal imaging can be used for breeding soundness evaluation of zebu bulls.

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