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Sperm morphometry in high-fertile and low-fertile Mehsana buffalo bulls

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Abstract
The present study was conducted to compare various sperm morphometric parameters between high-fertile and low-fertile Mehsana buffalo bull. A total of twelve Mehsana buffalo bulls were selected based on their conception rate (CR) recorded under the field progeny testing program and divided into two equal groups (high-fertile - CR $\geq$ 43% and low-fertile - CR $\leq$ 35%). To compare various sperm morphometric parameters, 72 frozen semen samples, six from each bull was randomly selected. Smears of frozen-thawed semen was stained with eosin-nigrosin stain and various morphometric parameters *viz.* sperm head length, sperm head width, width at base, mid-piece length, ellipticity, elongation, head shape and head area were recorded. The mean value of sperm head width (µm), mid-piece length (µm), elongation (%) and head shape were significantly ($p<0.05$) higher in high fertile bull as compared to low fertile bull. While, width at base (µm) and ellipticity were significantly ($p<0.05$) higher in low fertile bull as compared to high fertile bull. Moreover, the sperm head length and head area differed non-significantly between high and low fertile buffalo bulls.

Keywords: Buffalo bull, conception rate, high-fertile bull, low-fertile bull, sperm morphometry

Introduction
Agricultural business is a dominant in India in which livestock constitute a major portion. The country has the largest population of bovines including 192.50 million cattle and 109.9 million buffaloes (Anonymous, 2019) [3]. The domestic buffaloes contribute substantially in terms of milk, meat and hide to the national economy. According to the Central Institute for Research on Buffaloes' 2019-20 annual report, it contributes 91.82 million metric tonnes of milk to the agricultural economy. India is a source of some of the best riverine breeds of buffaloes including Murrah, Nili-Ravi, Mehsana and Jafarabadi. However, the major constraints in most of the buffalo breeds have their poor reproductive efficiency.

Artificial insemination (AI) has been most popular in veterinary practices and has been universally accepted for the genetic improvement of the animals (Chaudhari *et al.*, 2007) [9]. AI is one of the earliest assisted reproductive technologies introduced since many years; although, the coverage of AI in buffaloes is less as compared to cattle owing to the poor conception rate with frozen semen (Kumaresan and Ansari, 2001) [14]. Although numbers of factors affecting the semen *viz.*, scrotal circumference (Chaudhari *et al.*, 2006) [8], age (Atara *et al.*, 2018) [4] season (Atara *et al.*, 2019 [6], Patel *et al.*, 2020 [15]), body weight (Atara *et al.*, 2020) [5]; freezing-thawing process has maximum impact on sperm quality with decisive changes in sperm plasma membrane integrity. Ultimately, cryopreservation can have a detrimental effect on the normal physiology of sperm, resulting into damage and modifications and finally death of the sperm, thereby reducing freeze-thawed quality parameters of semen (Bailey *et al.*, 2000) [7]. Although cryopreservation is the best method to preserve gametes for long-term use, due to its detrimental effect to sperm integrity by altering the membrane structure-function and cell metabolism (Hammerstedt *et al.*, 1990) [12], proper evaluation of frozen-thawed semen is very crucial aspect of frozen semen technology to achieve desirable conception rate in the field. Leading to a significant decline in semen quality during freezing-thawing process and alterations in sperm structure, sperm morphometry (Chaudhary *et al.*, 2022) [10] is one of the important criteria among various semen evaluation tests to study the quality of frozen-thawed sperm. Combining head shape and sperm morphometry, as well as other objective traits into an overall fertility index, could expect sires to be ranked based on their ability to fertilise (Aggarwal *et al.*, 2007) [1]. Considering the likely influence of sperm morphometry to the evaluation of sire fertility, the present study was conducted to compare the morphometry of high-fertile and low-fertile cryopreserved Mehsana bull spermatozoa.

Material and Methods

Selection and grouping of bulls

Total twelve Mehsana buffalo bulls from the Dama semen production unit, Banas Dairy were selected based on their conception rate (CR) recorded under the field progeny testing program, led by Banaskantha District Co-Operative Milk Producers' Union Ltd., Palanpur. The selected Mehsana buffalo bulls were divided into two equal groups (high-fertile - CR ≥ 43% and low-fertile - CR ≤ 35%).

Sperm morphometry

To compare various sperm morphometric parameters, 72 frozen semen samples, six from each bull was randomly selected. From each frozen-thawed semen sample, a smear was prepared on a pre-warmed clean glass slide by gentle mixing of a drop of frozen-thawed semen and a drop of pre warmed (37 °C) eosin-nigrosin stain. All the stained smears were examined under a microscope with oil immersion and evaluated for various morphometric parameters using image analysis software (Zen 2012, Carl Zeiss Microscopy GmbH). A total of 720 spermatozoa were measured for various morphometric parameters *viz*., sperm head length (at the longest portion), sperm head width (at the widest portion), width at base and mid-piece length. While, ellipticity, elongation, head shape and head area of sperm were calculated using following formulas (Rana *et al*., 2020) [16].

Ellipticity = (L-W)/ (L+W)
Elongation (%) = W/L × 100
Head shape = Width/Length ratio
Head area = $1.5-0.081\times B^2+0.64\times W\times L$
Where,
B = Width at base (µm)
L = Sperm head length (µm)
W = Sperm head width (µm)

Statistical analysis

The statistical difference between the groups were seen using one-way independent t-test and data were presented as mean ± S.E. Significance was set at 95%. Data analysis was done with SPSS 20 software.

Results

The mean values of various sperm morphometrical parameters are shown in table 1. The mean value of sperm head width (µm), mid-piece length (µm), elongation (%) and head shape were significantly ($p<0.05$) higher in high fertile bull (4.68±0.01, 11.85±0.05, 63.57±0.00 and 0.64±0.00, respectively) as compared to low fertile bull (4.62±0.02, 11.56±0.04, 62.45±0.00 and 0.62±0.00, respectively). While, width at base (µm) and ellipticity were significantly ($p<0.05$) higher in low fertile (2.66±0.02 and 0.23±0.00, respectively) bull as compared to high fertile bull (2.59±0.02 and 0.22±0.00, respectively). Moreover, the sperm head length (7.37±0.02 µm, 7.41±0.02 µm) and head area (23.01±0.09 µm^2, 22.84±0.12 µm^2) differed non-significantly between high and low fertile buffalo bulls, respectively.

The overall mean values of head length (µm), head width (µm), width at base (µm), mid-piece length (µm), ellipticity, elongation (%), head shape and head area were 7.39±0.04, 4.65±0.03, 2.62±0.04, 11.71±0.10, 0.23±0.00, 63.02±0.00, 0.63±0.00 and 22.92±0.23, respectively in present study.

Table 1: Sperm morphometry (Mean ± SE) of frozen-thawed semen in high fertile and low fertile buffalo bull

Fertility group	Head length (µm)	Head width (µm)	Width at base (µm)	Mid-piece length (µm)	Ellipticity	Elongation (%)	Head shape	Head Area (µm^2)
HF (n = 360)	7.37±0.02^A	4.68±0.01^B	2.59±0.02^A	11.85±0.05^B	0.22±0.00^A	63.57±0.00^B	0.64±0.00^B	23.01±0.09^A
LF (n = 360)	7.41±0.02^A	4.62±0.02^A	2.66±0.02^B	11.56±0.04^A	0.23±0.00^B	62.45±0.00^A	0.62±0.00^A	22.84±0.12^A
Overall	7.39±0.04	4.65±0.03	2.62±0.04	11.71±0.10	0.23±0.00	63.02±0.00	0.63±0.00	22.92±0.23
p-value	0.105	0.006	0.005	0.001	0.001	0.001	0.001	0.247

Means bearing different subscripts (A, B) differ significantly within column ($p<0.05$).

Discussion

In agreement with the present study, Kumar *et al*. (2014) [13] found significantly higher mean value of sperm head width in high fertile group as compared to low fertile group. In country with the present findings, they reported significantly higher mean value of sperm head width in high fertile group as compared to low fertile group. Gravance *et al.* (2009) [11] reported non-significant difference in sperm head length, width and area between the two groups.

The overall mean value of sperm head length (7.39 µm) observed in present study was in accordance with Anilkumar *et al.* (2017) [2] in Toda buffalo bull and Roy *et al.* (2008) [17] in Murrah buffalo bull. However, Rana *et al.* (2020) [16] found lower value of sperm head length (7.24 µm) in Murrah buffalo bull. While, Aggarwal *et al*. (2007) [1] found higher value of sperm head length in Mehsana, Murrah, Nili-ravi and Surti (9.98, 9.69, 9.33 and 9.97, respectively) buffalo bull. The overall mean value of sperm head width (4.65 µm) observed in present study was higher than the values reported by Rana *et al.* (2020) [16] and lower than the Anilkumar *et al.* (2017) [2] and Aggarwal *et al*. (2007) [1] in various breeds of buffalo bulls. The mean value of sperm width at base (2.62 µm) reported in present study were in agreement with Rana *et al.* (2020) [16] and higher than reported by Roy *et al.* (2008) [17]. The mean value of mid-piece length (11.71 µm) reported in present study was lower than the value reported by Rana *et al.* (2020) [16]. The overall mean value of elongation (63.02%) reported in present experiment was higher than Rana *et al.* (2020) [16] and in accordance with Anilkumar *et al.* (2017) [2]. The overall mean value of head shape observed in present study was higher than the value reported by Rana *et al.* (2020) [16]. The overall mean value of head area (µm^2) recorded in present study was higher than Rana *et al.* (2020) [16] and lower than Anilkumar *et al.* (2017) [2] and Aggarwal *et al.* (2007) [1].

Conclusion

Among sperm morphometric parameters head width, width at base, mid-piece length, ellipticity and elongation were significantly differed between high fertile and low fertile bulls.

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