

Study the Morphometry of Fresh and Cryopreserved Murrah Bull Spermatozoa

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Abstract Artificial insemination with frozen – thawed spermatozoa was introduced in most of the developing countries more than three decades ago, yet it has not been successfully applied in large scale (Anzar *et al.*, 2003). More than 50% spermatozoa are usually injured by the cryopreservation process (Watson, 1995) injuries due to cryopreservation and more likely due to the increased solute concentration and the formation of intracellular and extracellular ice crystals during cryopreservation (Mazur, 1984) leading to a significant decline in semen quality and alterations in sperm morphometrix. Therefore, the present study was designed to study cryopreservation of buffalo semen and the effect of semen cryopreservation on determinant like semen fertility viz. sperm morphometry. Forty-eight semen samples collected from eight Murrah buffalo bulls maintained at Central semen station, Bhopal were included for the study of physic-morphological characters of neat and cryopreserved semen. Various measurements of the sperm head (length, width, base, ellipticity, elongation, head shape and area) and sperm tail (length of Midpiece and length of tail) were recorded in neat and cryopreserved semen. The average sperm measurements recorded were 7.24 ± 0.06 , 4.26 ± 0.05 , 2.65 ± 0.79 , 11.92 ± 1.53 , 43.12 ± 2.79 , 61.97 ± 0.50 , 25 ± 0.07 , 19.13 ± 2.58 , 0.59 ± 0.01 , and 59.07 ± 0.94 , respectively. There was a significant ($P < 0.05$) decline of sperm head length, head width, midpiece length, tail length, total length and head area during the process of cryopreservation. However, no significant difference between width at base, ellipticity, head shape and elongation of the spermatozoa in neat and cryopreserved semen was recorded.

Keywords Cryopreservation, Murrah, Morphometry

1. Introduction

Buffalo has been an important animal species of Indian subcontinent since prehistoric time. It contributes

substantially in terms of milk, meat and hide to the national economy. The world buffalo population is 152 million (FAO, 2003) distributed in 40 countries as 20 recognized breeds and this wonderful animal is slowly becoming recognized as the world's most important animal species especially in the countries where it exist in large numbers. India is a source of some of the best riverine breeds of buffaloes. Murrah, Nili-Ravi and Surti enjoy a dominant position among breeds noted for milk production. Bhadawari is reported to have high milk fat and Jaffarabadi is the heaviest of all the Indian buffalo breeds. The buffalo breeds of Indian subcontinent play important roles as producers of milk, draught power, dung and other value-added products. However, one of the major constraints for full exploitation of the productive potential of buffalo has been its inherently low reproductive efficiency. Artificial insemination with frozen – thawed spermatozoa was introduced in most of the developing countries more than three decades ago, yet it has not been successfully applied in large scale (Anzar *et al.*, 2003). Advances in cryopreservation of bull spermatozoa have not kept pace with the advances that occurred in other reproductive technologies. More than 50% spermatozoa are usually injured by the cryopreservation process (Watson, 1995) injuries due to cryopreservation are more likely due to increasing solute concentration and the formation of intracellular and extracellular ice crystals during cryopreservation (Mazur, 1984) leading to significant decline in semen quality and alterations in sperm morphometrix. Only minimal changes in protocols for processing and freezing bull semen have been implemented in the past 20 years. This limited rate of progress is due to lack of complete understanding of the factors and interactions that alter spermatozoa viability upon processing, freezing and thawing. Straw are highly sensitive to temperature changes for their high surface to volume ratio. Thawing procedure is very important in terms of its impact on the reanimation of spermatozoa. Several trials have been carried out to decide most ideal

thawing procedure for frozen semen. The post thaw fertilizing ability of spermatozoa is greatly affected by protocols such as thawing temperature and duration. Considering the importance of minimizing damage to the spermatozoa during cryopreservation process and need of accurate assessment of the sperm fertility in implementation of any artificial insemination programme, the present study was conducted with the following objective to study the morphometrix of fresh and cryopreserved Murrah bull spermatozoa.

2. Materials and Methods

2.1. Experimental Design

The present study was conducted in the Department of Animal Reproduction, Gynaecology & Obstetrics, College of Veterinary Science & Animal Husbandry, Mhow and central semen station, Bhopal. Eight Murrah buffalo bulls maintained under identical and optimal conditions of feeding and management in Central Semen Station, Bhopal were included in the study.

2.2. Method of Sperm Collection and Preservation

Fourty eight semen samples (six samples from each Murrah buffalo bull) were collected at Central Semen Station, Bhopal by artificial vagina method as described by Tomar (1997) in the morning hour, at 72 hours interval. Immediately after collection, neat semen evaluation was done and semen was subjected to examination filling, sealing, equilibration and cryopreservation as per the standard procedure using automated filling and sealing machine, cold handling cabinet and programmable biofreezer.

2.3. The following Parameter was Studied in Fresh and Frozen Semen

Sperm Morphometrix

Sperm morphometrix was studied in each sample to study the difference of sperm morphometrix in neat and frozen semen. Sperm were examined (1000 X magnification) with a Trinocular Research Phase Contrast Microscope (Manufactured by Leica Microsystems Shanghai, China) fitted with a high resolution digital CCD camera (still image resolution = 768 _ 576 pixels) and analyzed with imaging software (Leica DME Images plus 2.0; Leica DME China Group, Ltd., Shanghai). The system was first calibrated with images of standard length for known magnifications and measurement accuracy of 0.1 mm. Various measurements of the sperm head (length, width, base, ellipticity, elongation, head shape and area) and sperm tail (length of Midpiece and length of tail) were recorded.

(i) Sperm head length

(ii) Sperm head width (At the widest portion)

(iii) Width at base

(iv) Midpiece length

(v) Tail length

(vi) Total length

(vii) Head area (A) = $1.5 - 0.081 \times B2 + 0.64 \times w \times L$

(viii) Ellipticity $(L-W)/(L+W)$

(ix) Elongation $W/L \times 100$ (%)

(x) Head shape (Width: Length ratio)

2.4. Statistical Analysis

The data were subjected to statistical analysis as per methods described by Snedecor and Cochran (1994). Pair 't' test was applied to compare different characteristics of neat and frozen.

3. Results

3.1. Sperm Head Length

The head length of the eight Murrah bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII were 7.30 ± 0.682 , 7.35 ± 0.04 , 7.46 ± 0.05 , 7.65 ± 0.05 , 7.38 ± 0.05 , 7.63 ± 0.04 , 7.37 ± 0.04 and 7.60 ± 0.05 with an overall average of 7.47 ± 0.05 μ . Significantly ($P < 0.05$) higher sperm head length was recorded in bull MB IV, VI & VIII as compared to the other bulls in the present study.

3.2. Sperm Head Width

The average head width (mean \pm SE) of the Murrah bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII were 4.33 ± 0.05 , 4.38 ± 0.05 , 4.58 ± 0.04 , 4.39 ± 0.05 , 4.43 ± 0.04 , 4.45 ± 0.05 , 4.53 ± 0.05 and 4.33 ± 0.04 μ , respectively. The overall average of the eight bulls was 4.43 ± 0.04 μ . There was significant ($P < 0.05$) between bulls and between ejaculates variations of sperm head width in the present study. Bulls MB III and MB VII recorded significantly ($P < 0.05$) higher head width as compared to the other bulls in the current study.

3.3. Width at Base

The width at base (mean \pm SE) of the Murrah bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII recorded in the present study was 2.83 ± 0.06 , 2.57 ± 0.04 , 2.62 ± 0.05 , 2.57 ± 0.04 , 2.70 ± 0.10 , 2.62 ± 0.06 , 2.67 ± 0.47 and 2.56 ± 0.53 , respectively with an overall average of 2.66 ± 0.07 μ . Significantly ($P < 0.05$) higher width at base was recorded in bull MB I as compared to the other bulls in the present study.

3.4. Sperm Midpiece Length

The midpiece length of the Murrah buffalo bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII in the present study was 12.21 ± 0.18 , 12.22 ± 0.08 ,

12.2±0.18, 12.16±0.08, 11.73±0.21, 12.16±0.17, 12.57±0.08 and 12.06±0.18 μ , respectively. The overall average of midpiece length of all the eight Murrah bulls was 12.17±0.14 μ . In the present study significantly lower ($P<0.05$) sperm midpiece length was recorded in bull MB V as compared to MB I, II, III, IV and VII. No significant difference was, however, recorded between bulls MB V, VI and VIII.

3.5. Sperm Tail Length

The tail length (mean±SE) of the Murrah bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII recorded in the present study was 44.18±0.31, 43.70±0.33, 43.75±0.22, 43.59±0.25, 43.26±0.24, 43.48±0.26, 45.04±0.30, 42.69±0.58 and 43.63±0.43 μ , respectively with an overall average of 43.63±0.43 μ . The mean length tail of Murrah buffalo bulls spermatozoa in the present study was recorded to be significantly ($P<0.05$) higher in MB VII as compared to the other bulls. There was significant ($P<0.05$) difference between sperm tail length of MB I and MB VIII.

3.6. Total Sperm Length

The total sperm length of the Murrah buffalo bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII in the present study was 63.73±0.36, 63.28±0.62, 62.82±0.70, 63.41±0.26, 62.38±0.07, 63.27±0.32, 65.01±0.34 and 62.36±0.63 μ , respectively. The overall average of total sperm length of all the eight Murrah bulls was 63.28±0.36 μ . Bull MB VII showed significantly

($P<0.05$) higher total sperm length as compared to the other bulls in the present study.

3.7. Sperm Ellipticity

The mean±SE of ellipticity of spermatozoa of the eight Murrah buffalo bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII recorded in the present study was 0.26±0.07, 0.25±0.05, 0.24±0.05, 0.27±0.06, 0.25±0.01, 0.26±0.01, 0.24±0.33 and 0.27±0.01, respectively. The overall average of the eight bulls recorded was 0.26±0.02. In the present study significant difference ($P<0.05$) between bulls and ejaculate differences in ellipticity of spermatozoa was recorded. Bulls MB IV and MB VIII showed significantly higher ellipticity as compared to bulls MB II, III, V and VII. No significant difference of ellipticity was, however, recorded between bulls MB I, MB IV, MB VI and MB VIII.

3.8. Sperm Head Area

The head area of the Murrah buffalo bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII recorded in the present study was 21.67±0.33, 21.94±0.28, 23.21±0.27, 22.82±0.30, 22.23±0.23, 23.05±0.29, 22.70±0.44 and 22.36±0.01, square micron respectively. The overall average of Head area of spermatozoa of the eight Murrah bulls was 22.49±0.29 square micron. Significantly ($P<0.05$) higher sperm head area was recorded in the bulls MB III and VI as compared to the bulls MB I, II and V.

Table 1. Sperm morphometry parameters of neat semen

Bulls	MB I	MB II	MB III	MB IV	MB V	MB VI	MB VII	MB VIII	Overall average
Head Length (μ)	7.30 ^a ±0.682	7.35 ^a ±0.04	7.46 ^{ac} ±0.05	7.65 ^b ±0.05	7.38 ^a ±0.05	7.63 ^b ±0.04	7.37 ^a ±0.04	7.60 ^{bc} ±0.05	7.47 ±0.05
Head Width (μ)	4.33 ^a ±0.05	4.38 ^a ±0.05	4.58 ^{bc} ±0.04	4.39 ^{ac} ±0.05	4.43 ^{ac} ±0.04	4.45 ^{ac} ±0.05	4.53 ^{bc} ±0.05	4.33 ^a ±0.04	4.43 ±0.04
Width at Base (μ)	2.83 ^a ±0.06	2.57 ^{bc} ±0.04	2.62 ^{bc} ±0.05	2.57 ^{bc} ±0.04	2.70 ^{ac} ±0.10	2.62 ^{bc} ±0.06	2.67 ^{ac} ±0.47	2.56 ^{bc} ±0.53	2.66 ±0.07
Midpiece length (μ)	12.21 ^{ad} ±0.18	12.22 ^{ad} ±0.08	12.2 ^{ad} ±0.18	12.16 ^{ad} ±0.08	11.73 ^{bc} ±0.21	12.16 ^{ad} ±0.17	12.57 ^a ±0.08	12.06 ^{dc} ±0.18	12.17 ±0.14
Tail Length (μ)	44.1 ^{ad} ±0.31	43.70 ^{ac} ±0.33	43.75 ^{ac} ±0.22	43.59 ^{ac} ±0.25	43.26 ^{ac} ±0.24	43.48 ^{ac} ±0.26	45.04 ^d ±0.30	42.69 ^{bc} ±0.58	43.63 ±0.43
Total length (μ)	63.73 ^a ±0.36	63.28 ^a ±0.62	62.82 ^a ±0.70	63.41 ^a ±0.26	62.38 ^a ±0.07	63.27 ^a ±0.32	65.01 ^a ±0.34	62.36 ^a ±0.63	63.28 ±0.36
Ellipticity	0.26 ^{ac} ±0.07	0.25 ^a ±0.05	0.24 ^a ±0.05	0.27 ^{bc} ±0.06	0.25 ^a ±0.01	0.26 ^{ac} ±0.01	0.24 ^a ±0.33	0.27 ^{bc} ±0.01	0.26 ±0.02
Head Area	21.67 ^a ±0.33	21.94 ^{ac} ±0.28	23.21 ^{bd} ±0.27	22.82 ^{bc} ±0.30	22.24 ^{ac} ±0.23	23.06 ^{bd} ±0.29	22.70 ^{ad} ±0.44	22.37 ^{ad} ±0.01	22.49 ±0.29
Head Shape	0.59 ^{adc} ±0.08	0.60 ^{adc} ±0.06	0.61 ^a ±0.06	0.57 ^{bc} ±0.07	0.60 ^{ad} ±0.07	0.58 ^c ±0.01	0.61 ^a ±0.06	0.57 ^{bc} ±0.08	0.59 ±0.07
Elongation	59.48 ^{ace} ±0.85	59.79 ^{ace} ±0.71	61.61 ^a ±0.66	57.51 ^{bc} ±0.75	60.18 ^{ace} ±0.71	58.38 ^{ce} ±0.69	61.56 ^a ±0.08	57.24 ^{dce} ±0.86	59.49 ±0.78

Different superscripts within a row indicates significant difference ($P<0.05$)

3.9. Sperm Head Shape

The head shape (mean±SE) of the Murrah bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII recorded in the present study was 0.59±0.08, 0.59±0.06, 0.61±0.06, 0.57±0.07, 0.61±0.07, 0.58±0.01, 0.61±0.06 and 0.57±0.08, respectively with an overall average of 0.59±0.07. There was significant (P<0.05) between bulls and ejaculate variation in the sperm head shape in the present study.

3.10. Sperm Head Elongation

The Elongation of spermatozoa in the semen of eight

bulls in the present study averaged 59.66±0.85, 59.79±0.71, 61.61±0.66, 57.50±0.75, 60.18±0.71, 58.38±0.69, 61.56±0.08 and 57.24±0.86, respectively for MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII bulls. The average concentration of the sperm of all the eight bulls was 59.49±0.78. Significantly (P<0.05) higher values for sperm head elongation was recorded in bulls MB III and MB VII as compared to MB IV, VI and VIII. No significant difference between bulls MB I, II, IV, V, VI and VIII was, however, recorded in the present study

3.11. Cryopreserved Semen

Table 2. Sperm morphometric parameters of cryopreserved semen

Bulls	MB I	MB II	MBIII	MB IV	MB V	MB VI	MB VII	MB VIII	Overall average
head Length	7.07±0.09 ^a	7.26±0.55 ^a	7.22±0.05 ^a	7.26±0.05 ^a	7.05±0.06 ^a	7.36±0.08 ^b	7.31±0.06 ^b	7.35±0.05 ^b	7.24±0.06
head Width	4.23±0.05 ^{ab}	4.19±0.04 ^a	4.44±0.06 ^b	4.22±0.05 ^a	4.28±0.06 ^{ab}	4.19±0.05 ^a	4.31±0.05 ^{ab}	4.25±0.05 ^{ab}	4.26±0.05
Width at Base	2.59±0.39 ^{ab}	2.51±0.04 ^a	2.75±0.04 ^{ab}	2.53±0.09 ^{ab}	2.66±0.04 ^{ab}	2.57±0.1 ^{ab}	2.75±0.08 ^b	2.5±0.05 ^a	2.65±0.79
Midpiece length	12.07±0.09 ^a	11.94±0.11 ^{ab}	11.72±0.20 ^{bc}	11.81±0.08 ^{ab}	11.48±0.47 ^b	12.10±0.10 ^{ac}	12.06±0.08 ^{ac}	11.87±0.09 ^{ab}	11.92±1.53
Sperm Tail Length	43.03±0.34 ^{ab}	43.26±0.33 ^a	43.21±0.36 ^{ab}	42.70±0.25 ^{ab}	43.52±0.32 ^a	42.15±0.34 ^b	43.70±0.36 ^a	43.31±0.41 ^{ab}	43.12±2.79
Total sperm length	62.16±0.39 ^a	62.53±0.3 ^a	62.17±0.4 ^a	61.77±0.3 ^a	61.91±0.4 ^a	61.70±0.3 ^a	61.53±0.36 ^a	63.01±0.61 ^a	61.97±0.5
head Ellipticity	0.24±0.09 ^a	0.26±0.06 ^b	0.24±0.01 ^a	0.26±0.04 ^b	0.24±0.01 ^a	0.27±0.06 ^b	0.26±0.01 ^b	0.27±0.01 ^b	0.25±0.07
head area	18.56±0.36 ^a	18.98±0.28 ^{ab}	19.57±0.31 ^b	19.10±0.29 ^{ab}	18.71±0.36 ^{ab}	19.27±0.2 ^{ab}	19.35±0.33 ^{ab}	19.32±0.39 ^{ab}	19.13±2.58
head Shape	0.60±0.01 ^a	0.57±0.07 ^b	0.60±0.01 ^a	0.58±0.07 ^{ab}	0.61±0.01 ^a	0.56±0.01 ^b	0.59±0.01 ^{ab}	0.57±0.01 ^b	0.59±0.01
head elongation	60.73±0.31 ^{ac}	57.93±0.76 ^c	60.93±0.83 ^a	58.14±0.71 ^c	60.42±1.01 ^{ac}	56.55±0.75 ^b	59.21±0.84 ^{ac}	57.93±0.8 ^c	59.07±0.94

Different superscripts within a row indicates significant difference (P<0.05).

Table 3. Sperm morphometric parameters of neat and cryopreserved semen

Morphometric parameters	Neat Semen	Post- thaw
Head Length (μ)	7.47±0.05 ^a	7.24±0.06 ^b
Head Width (μ)	4.43±0.04 ^a	4.26±0.05 ^b
Width at Base (μ)	2.66±0.07 ^a	2.65±0.79 ^a
Midpiece length (μ)	12.17±0.14 ^a	11.92±1.53 ^b
Tail Length (μ)	43.63±0.43 ^a	43.12±2.79 ^b
Total length (μ)	63.28±0.36 ^a	61.97±0.55 ^b
Ellipticity	0.26±0.02 ^a	0.25±0.07 ^a
Head Area	22.49±0.29 ^a	19.13±2.58 ^b
Head Shape	0.59±0.07 ^a	0.59±0.01 ^a
Elongation	59.49±0.78 ^a	59.07±0.94 ^a

Different superscripts within a row indicates significant difference (P<0.05)

3.12. Correlation between Sperm Morphometry Parameters

The correlation between the different sperm morphometry parameters of Murrah semen is summarized in Table 1 and 2, on the basis of calculated correlation coefficient in neat as well as cryopreserved semen.

The neat semen tail length was significantly negatively correlated with head length ($r = -0.5259$) and significantly positively correlated with head width ($r = 0.4720$), width at base ($r = 0.4026$) and midpiece length ($r = 0.7870$). The head length was also significantly negatively correlated with width at base ($r = -0.5503$) and midpiece length ($r = -0.3935$). The head width was also significantly positively ($r = 0.3378$) with sperm midpiece length. A highly significant correlation of total sperm length with head length ($r = 0.3349$), mid piece length ($r = 0.9097$) and tail length ($r = 0.960$) was also recorded in the present study. Sperm cell ellipticity also had significantly negative correlation with sperm head length ($r = -0.540$) and width at base ($r = -0.3211$) and significant positive correlation with head width ($r = 0.7303$) and midpiece length ($r = 0.9827$) was also recorded in the present study. There was significant positive correlation of sperm midpiece length with sperm ellipticity ($r = 0.9827$) in neat semen. There was highly significant positive correlation of sperm head area with head length ($r = 0.6348$) and head width ($r = 0.7333$) and significant negative ($r = -0.5630$) correlation with width at base. Midpiece length, tail length, total sperm length and Ellipticity of spermatozoa, however, did not significantly correlate with head area. A significant positive correlation of sperm head shape with head width ($r = 0.7333$), Ellipticity ($r = 0.530$) and head area ($r = 0.566$) and a significant negative correlation of sperm head length ($r = -0.6979$) with sperm head shape.

Sperm cell elongation was significantly negatively correlated with head length ($r = -0.7234$) and ellipticity ($r = -0.529$) and significantly positively correlated with head width ($r = 0.7333$), head shape ($r = 0.675$) and head area ($r = 0.4860$). No significant correlation of sperm elongation was, however, observed with width at base, midpiece length, tail length, total sperm length and head area.

As evident from Table 2 and 3 similar trends were also observed in post thaw semen

4. Discussion

4.1. Neat Semen Sperm Morphometric Parameters

Sperm morphometry, in combination with other objective traits, can be useful for developing a fertility index. Particularly, sperm head abnormalities have been associated with lower fertility (Saake and White, 1972; Phetudomsinsuk *et al.*, 2008). Routine sperm head morphology assessment are highly subjective and sperm

morphometry promises enormous potential for evolving as a possible objective and accurate tool for prediction of bull fertility.

4.2. Sperm Head Length

The sperm head length (mean \pm SE) recorded in the present study ($7.47\pm 0.05 \mu$) was in compliance to several other reports in the past (Aggrawal *et al.*, 2007; Roy *et al.*, 2008). Comparatively higher sperm head length was recorded in *Bos indicus* (8.3μ) and *Bos taurus* (8.9μ) in the past (Belleti *et al.*, 2005). Ciftci and Zulkadir (2010) also recorded longer sperm heads in HF bulls (14.25μ). These differences may be attributed to differences in species and breed, different managemental conditions, different agro-climatic conditions and different season of study. There was significant between bull variation of sperm head length in the present study. The variation may be due to genetic variation in different populations by selection (Saini, 1994).

4.3. Sperm Head Width

The average overall sperm head width of 4.43 ± 0.04 was recorded in the present study with significant ($P < 0.05$) variation between bulls and ejaculates. The mean sperm head width recorded in the present study is in fair compliance with several findings in the past (4.41μ : Ali *et al.*, 1976; 4.91μ : Kumar *et al.*, 1977; 4.75μ : Aggrawal *et al.*, 2007; 4.91μ : Roy *et al.*, 2008). Lesser values for water buffalo sperm head width has also been recorded in the past (2.3μ : Fischar and Gunzel, 1978). Fairly comparable values for cattle spermatozoa have been recorded in *Bos taurus* cattle in the past (4.71μ : Saini, 1994; 4.39μ : Grevance *et al.*, 1998; 4.5μ : Belleti *et al.*, 2005) suggesting similarity in sperm head width of cattle and buffalo spermatozoa. The significant ($P < 0.05$) difference between bulls recorded in the present study is in compliance with the findings of Katyari *et al.*, 1989, who recorded significant between breed, males and slides differences in his study on Murrah, Surti and Mehsana bulls. Similar between bull variations have also been recorded in buffalo (Biswas, 1976; Pant and Mukherjee, 1973; Aggrawal, 2007), Cattle (Pandya *et al.*, 1985; Saini, 1994) and bucks (Das and Sidhu, 1975; Kolachhapati, 1987). However, contrary results have also been reported by Ali (1976) and Mohan *et al.* (1981). The between bulls differences recorded in the present study and non compliance with few reports in the past may be due to the different genetics of bulls in these studies.

4.4. Width at Base

The overall mean \pm SE of width at base ($2.66\pm 0.07 \mu$) recorded in the present study is fairly comparable to the finding of 2.47μ recorded in the past (Roy *et al.*, 2008).

Limited literature is present in buffalo bulls for comparison of our results. In cattle smaller width at base (1.966μ) has been recorded in the past (Saini, 1994) suggesting smaller head base in cattle as compared to buffaloes. In the present study significant ($P < 0.05$) between bulls and between ejaculate variation was recorded. Similar between bull differences for this trait was also recorded in the past for buffalo (Deka, 1983), pigs (Batabyal, 1976), goat (Kolachhapati, 1987) and cattle (Saini, 1994). Whereas Bhusan (1989) reported no significant differences between bovine spermatozoa width at base. These variations between bulls might be due to either genetic or physiological factors governing spermatozoan growth. The nutrients in the germ cells are constant for all animals. Some germ cells have better growth potentiality, as determined by their haploid genotype, give larger spermatozoa while a low potential one give small spermatozoa.

4.5. Midpiece Length

The midpiece length recorded in the present study $12.17 \pm 0.14 \mu$ is in fair comparison to the 11.82μ (Rajawar and Mukherjee, 1969). Similar midpiece length has also been recorded in Haryana bulls (Saini, 1994). Significant differences between bulls and ejaculates have been observed in the present study which might be ascribed to the findings of Ciftci and Zulkadir (2010) that midpiece length is heritable and varies among the bulls within the same species. Similar between bull differences has also been reported in cattle (Pandya, 1985; Saini, 1994) and buffaloes (Mukherjee and bandhopadyay, 1982; Deka, 1983) in the past. The differences in spermatozoan attributes among bulls may be attributed to genetic differences in the physiological mechanism involved in the formation of spermatozoa. Between ejaculate variation in sperm midpiece length might be due to the fact that sperm midpiece also differs in the ejaculates of the same bull in different times.

4.6. Sperm Tail Length

A sperm tail length of $43.63 \pm 0.43 \mu$ was recorded in the present study. Similar finding were recorded by Vekataswamy and Vedanayagan, 1962, Roy *et al.*, 2008 (56.14μ) and Aggrawal *et al.* 2007 (57.02μ), however, recorded slightly greater sperm tail length in Murrah bulls. The differences may be due use of bulls of different genetic make up and different managemental and agroclimatic conditions. Significant ($P < 0.05$) between bulls variation have also been recorded in sperm tail length in the present study which may be due to use of bulls of different age groups (Aggrawal *et al.*, 2007).

4.7. Total Sperm Length

The total sperm length of $63.28 \pm 0.36 \mu$ recorded in the present study correspond well with the earlier reports in

Murrah bulls (63.42μ : Vekataswamy and Vedanayagan, 1962; 63.73μ : Roy *et al.*, 2008). Greater total sperm length, however, was recorded in Murrah (Aggrawal *et al.*, 2007) and Surti (Kodagali *et al.*, 1973) bulls in the past. Bull MB VII had longest spermatozoa as compared to other bulls in the experiment. Differences may be due to use bulls of different genetic makeup and age group.

4.8. Sperm Head Ellipticity

The sperm ellipticity recorded in the present study was 0.26 ± 0.02 in the present study was fairly comparable with the findings of Roy *et al.*, 2008 (0.21). The ellipticity of buffalo bulls recorded in the present study was lesser than that recorded in *Bos indicus* (0.30) and *Bos taurus* (0.32) spermatozoa (Belletti *et al.*, 2005). Similarly, higher ellipticity has also been recorded in bulls (Ciftci and Zulkadir, 2010). This is indicated by more elongated (elliptical) sperm heads and smaller length: width ratios. Significant ($P < 0.05$) between bull variation was recorded in sperm head ellipticity of Murrah bulls in the present study. Similar between animals/ slide variations of sperm biometrics parameters have also been recorded in the past (Katyar, 1989) in buffalo bulls.

4.9. Sperm Head area

The sperm head area (mean \pm SE) recorded in the present study ($22.49 \pm 0.29 \mu^2$) complied well with the findings of Roy *et al.*, 2008 in Murrah bulls and Kodagali *et al.*, 1973 in Surti bulls. Significantly, greater sperm head area in Murrah bulls was, however, also recorded in the past (Aggrawal *et al.*, 2007). These variations in head area may be due to genetic diversities in different populations. In *Bos taurus* ($32.9 \pm 3.6 \mu^2$) and *Bos indicus* ($30.1 \pm 3.6 \mu^2$) sperm head area (Beletti *et al.*, 2005) was also greater than the sperm head area recorded in the present study indicating larger size of the sperm head area in cattle as compared to Murrah buffaloes. Saini (1994) also recorded similar results in neat semen of Haryana bulls. Significant ($P < 0.05$) between bull difference recorded in the present study may also be due to difference in age and genetic make up of bulls used in the present study. Similar significant between bull variations were also recorded in cattle in the past (Saini, 1994) which might be due to variability of sperm nucleus in case of individual animals (Gledhill *et al.*, 1970). So the differences recorded in the present study are biological in nature.

Between bulls variations have also been recorded in other domestic animals like cattle (Mukherjee and Singh, 1966; Pandya, 1976), buffaloes (Pant and Mukherjee, 1972), goat (Das and Sidhu, 1975) and pigs (Batabyal, 1976).

4.10. Sperm Head Shape (Width: Length Ratio)

The head shape of 0.59 ± 0.07 was recorded in Murrah

bulls in the present study. Similar width length ratio was also recorded in the past (0.66: Tomar *et al.*, 1962; 0.62: Rajawar and Mukherjee, 1969; 0.61: Ali, 1976; 0.59: Aggrawal *et al.*, 2007). Higher, width:length ratio has also been recorded in cattle (Saini, 1994). Significant ($P < 0.05$) between bull variations was reported for this attribute in the present study. Similar findings were recorded in cattle (Pandya *et al.*, 1976; Bhusan, 1989), buffaloes (Ali, 1976, Mukherjee and Bandopadhyay, 1982, Deka, 1986) and goats (Kolachhapati, 1987). Non significant between bull differences was, however, also recorded in the past (Mohan *et al.*, 1981). The shape of the spermatozoa was not influenced by the external forces on the spermatid nucleus but the phenomenon was genetically controlled and dependent upon the condensation of DNA and protein of the nucleus.

4.11. Sperm Head Elongation

The sperm head elongation of 59.94 ± 0.78 was recorded in Murrah bulls in the present study. Similar findings were also recorded in the past (Tomar *et al.*, 1962; Rajawar and Mukherjee, 1969; Ali, 1976; Aggrawal *et al.*, 2007). Higher, sperm head elongation has also been recorded in cattle (Saini, 1994). Significant ($P < 0.05$) between bull variations was reported for this attribute in the present study. Similar findings were recorded in cattle (Pandya *et al.*, 1976; Bhusan, 1989), buffaloes (Ali, 1976, Mukherjee and Bandopadhyay, 1982, Deka, 1986) and goats (Kolachhapati, 1987).

4.12. Cryopreservation of Murrah Semen

Effect of Cryopreservation on Sperm Morphometry

The mean sperm head length, width, midpiece length, tail length, total sperm length, ellipticity, head area, head shape and elongation in cryopreserved semen of Murrah bulls compared fairly well with the findings of Aggrawal *et al.* (2007). The Sperm head length, head width, midpiece length, tail length, total sperm length and head area decreased significantly ($P < 0.05$) after freezing and thawing. The results in the present study are in compliance with the previous report in *Bos taurus* (Grevance *et al.*, 1998; Guillen *et al.*, 2007) and *Bos indicus* (Guillen *et al.*, 2007) bulls. A significant between bulls and between ejaculate difference was observed in sperm morphometric dimensions of frozen thawed semen of Murrah bulls in the present study. Similar differences were also observed in case of bovine (Grevance *et al.*, 1998) and caprine semen (Grevance *et al.*, 1997). The differences in morphometric dimensions between neat and cryopreserved spermatozoa observed in the present study may be explained by changes in sperm chromatin structure. The surface area of sperm heads tended to decrease after cryopreservation and thawing, which might be attributed to changes in sperm chromatin structure. It was hypothesized that

cryopreservation induces over condensation of sperm chromatin. A variety of spermatozoal injury, such as heat stress and cryopreservation can induce changes in sperm chromatin structure, resulting in acid induced denaturation (Blottner *et al.*, 2001). Few studies have also associated abnormal chromatin structure with abnormal sperm head morphology (Gledhill *et al.*, 1970; Grevance *et al.*, 1998) and reduced fertility. However, it is difficult to interpret whether morphometric dimensions are associated with abnormal chromatin structure and reduced fertility as reports for direct comparison of effect of cryopreservation on sperm morphometric dimensions of Murrah bulls are lacking. Acrosomal exocytosis and osmotic changes may also be reason for alteration in sperm morphometric dimensions during the process of cryopreservation (Thomas *et al.*, 1997; Blottner *et al.*, 2001; Estes *et al.*, 2003). One possible hypothesis for the reduction of sperm head dimensions of spermatozoa of all bulls may be an increase in number of spermatozoa with acrosomal damage or loss occurred during cryopreservation and thawing (Thomas *et al.*, 1997). The proportion of acrosome reacted spermatozoa is also significantly increased during the process of cryopreservation and thawing (Chauhan *et al.*, 1991). Cryopreservation may also be responsible for membrane damage of bovine spermatozoa (Valcarcel *et al.*, 1994). Because the present study did not deal with morphology of the nucleus and acrosome separately, the role of these individual component could not be assessed for causing decrease in bull sperm head morphometric dimensions. However, the process of cryopreservation led to increase in the number of dead spermatozoa with the loss of acrosomal and plasma membranes during cell death (Estes *et al.*, 2006). Therefore, decrease of sperm head dimensions in the present study could, at least, partially be explained by damage or loss to the acrosome and increase in percentage of acrosome reacted spermatozoa, as cited by Grevance *et al.*, 1998 and Guillen *et al.*, 2007.

Reduction in seminal characteristics after post thaw incubation might be due to damage to plasma membrane this damage might have occurred due to continuous accumulation toxic end product owing to increase oxidative metabolism resulting in increase concentration of peroxide formation in the medium with the increase in the number of dead spermatozoa (John *et al.*, 1979) with the increase in perportion of dead spermatozoa the toxicity of the medium might have increase which might have cause further damage to spermatozoa. Contradictory reports of increased sperm head dimensions after cryopreservation and thawing, however, has also been reported in the past (Grevance *et al.*, 1997). The differences may be due to differences in fixation technique, staining procedure, individual or species differences.

4.13. Correlation between Seminal Traits

In the present study a significant negative correlation of sperm tail length with sperm head length and significant

positive correlation with head width, width at base and midpiece length was recorded. Increased midpiece length, resulting from more or larger mitochondria, results in greater energy required to achieve higher swimming speeds. Longer tails are also required for greater swimming speeds. A significantly negative correlation of sperm head length with width at base and midpiece length and significant positive correlation of sperm head width with sperm midpiece length was also recorded in the present study. Similar positive correlation between sperm head length and midpiece length was also reported in porcine (Gill *et al.*, 2009). In contrast, a negative correlation of sperm head length with midpiece length has also been reported in the past (Humphries *et al.*, 2008). Reports suggesting no significant correlation of sperm midpiece length and sperm head length are, however, also on records (Malo *et al.*, 2006; Ciftci and Zulkadir, 2010). The highly significant positive correlation of total sperm length with head length, mid piece length and tail length recorded in the present study suggest that sperm with longer heads are associated with greater overall length.

The significant negative correlation of sperm head ellipticity with sperm head length and width at base and significant positive correlation with head width and midpiece length, in the present study are in compliance to the similar reports in porcine (Gill *et al.*, 2009) where a significantly negative correlation of sperm head size with Ellipticity was reported. There was highly significant positive correlation of sperm head area with head length and head width and significant negative correlation with width at base suggesting that sperm cells with longer and broader heads are associated with greater areas. Sperm cell elongation was significantly negatively correlated with head length and ellipticity and significantly positively correlated with head width, head shape and head area. Similar negative correlation of sperm cell ellipticity with elongations was also reported in porcine spermatozoa (Gill *et al.*, 2009). Whereas, few other studies suggest no correlation of sperm head dimensions with sperm cell ellipticity (Malo *et al.*, 2006; Ciftci and Zulkadir, 2010). Reports of similar studies in buffaloes are either lacking or are scanty, therefore, further studies are warranted to confirm or negate our findings.

5. Conclusions

In the present study, there was a significant difference between bull variations was recorded in neat semen head length, head width, width at base, midpiece length, tail length, total sperm length, ellipticity sperm head area, sperm head shape and elongation.

There was a significant difference between bull and between ejaculate variation in all the sperm morphometric parameters of the cryopreserved except variation in the total sperm length. There was significant decline of sperm

head length, head width, midpiece length, tail length, total length and head area during the process of cryopreservation. Sperm conception was significantly negatively correlated with head length, head width, Width at base, Tail length and total sperm length and positively correlated with Ellipticity, head area head shape and elongation.

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