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Study the Physico-Morphologic Parameters of Neat Cryopreserved Murrah Semen

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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. More than 50% spermatozoa are usually injured during the cryopreservation process. Injuries caused by cryopreservation are more likely due to increasing solute concentration and the formation of intracellular and extracellular ice crystals during cryopreservation [54] leading to 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. physico-morphologic. Forty eight semen samples collected from eight Murrah buffalo bulls maintained at Central semen station, Bhopal were included in the study for the study of physico-morphological characters of neat and cryopreserved semen. The overall average of the eight bulls for neat semen volume mass activity, progressive motility, live %, sperm concentration, head, midpiece, tail, and total sperm abnormalities was 2.89±0.14, 3.64±0.07, 71.56±0.37, 86.22±0.66, 963.92±38.51, 5.58±0.28, 2.18±0.32, 2.09±0.21 and 9.85±0.23 %, respectively. There was significant ($P<0.05$) between bulls and ejaculate variation in semen volume, sperm concentration, sperm midpiece and tail abnormalities. No significant differences between bulls as, however, recorded in sperm mass activity, progressive sperm motility, live sperm percent, sperm head and total sperm abnormalities.

Keywords Cryopreservation, Murrah, Physico-morphologic, Thawing

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 [38] 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 [6]. 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 [87] injuries due to cryopreservation are more likely due to increasing solute concentration and the formation of intracellular and extracellular ice crystals during cryopreservation [58] 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. Proteins are also known to be of great importance for the motility and the survival of spermatozoa during storage [76]. 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 physico-morphologic of fresh and cryopreserved Murrah bull spermatozoa.

2. Materials and Method

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.

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 [78] 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.

The Following Parameter was Studied in Fresh and Frozen Semen

Volume

Semen volume was recorded in graduated semen collection tube immediately after collection.

Mass Motility

The mass motility of fresh semen samples was observed as per the procedure described by [76]. A drop of neat semen was spread uniformly over pre-warmed, clean, dry, grease free glass slide and examined under low magnification of the microscope.

Individual Motility

The individual motility of fresh and frozen semen was expressed in terms of percentage of progressively motile spermatozoa as described by [90].

Sperm Concentration

Concentration of spermatozoa (million / ml) in fresh semen was determined by photoelectric colorimeter [87].

Live Sperm Percent

Live sperm percentage in fresh and frozen semen was estimated by Eosin –Nigrosin staining technique by [22]. Two hundred spermatozoa were examined for evaluation of live sperm percentage in each slide under at 100 X objective of a microscope.

Composition of stain:

Eosin –y:	0.4gm
Nigrosin:	10.0gm
Sodium citrate dehydrate:	2.90gm
Distilled water:	100ml
pH:	6.8 to 7.0
% live spermatozoa= Total no of. live spermatozoa / Total no. of spermatozoa X 100.	Statical analysis

The data were subjected to statistical analysis as per methods described by [23]. Pair ‘t’ test was applied to compare different characterstics of neat and frozen.

3. Results

Physico-morphological Parameters

Semen Volume

The average semen volume of the eight Murrah buffalo bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII was 3.06±0.33, 2.65±0.46, 3.7±0.57, 2.8±0.02, 3.5±0.34, 2.85±0.31, 2.93±0.46 and 2.03±0.23 ml, respectively with an overall average of 2.89±0.14 ml (Table 1). The semen volume of bull MB VIII differed significantly ($P<0.05$) from MB I and MB III. No significant difference between bulls MB I, MB II, MB III, MB IV, MB V, MB VI and MB VII and bulls MBII, MB IV, MB V, MB VI, MB VII and MB VIII was observed.

Table 1. Physico-morphological characteristics of Neat Murrah semen (Mean±SE)

Bulls …….. Seminal Attributes	MB I	MB II	MB III	MvB IV	MB V	MB VI	MB VII	MB VIII	Overall average
Volume (ml)	3.06a±0.33	2.65ab±0.46	3.7a±0.57	2.8ab±0.02	3.5ab±0.34	2.85ab±0.31	2.93ab±0.46	2.03b±0.23	2.89±0.14
Mass activity (0-4 scale)	3.73a±0.21	3.83a±0.16	3.83a±0.16	3.50a±0.22	3.33a ±0.21	3.83a ±0.16	3.83a ±0.16	3.66a ±0.21	3.64±0.07
Progressive motility (%)	71.66a±1.05	70.83a±0.83	72.50a±1.11	72.50a±1.11	70.83a±0.83	72.50a±1.11	70.83a±0.83	70.83a±0.83	71.56±0.37
Live sperm (%)	85.17a±2.99	83.50a±3.11	84.66a±3.11	88.00a±0.5	87.00a±1.36	87.67a±1.20	89.00a±1.00	84.83a±1.70	86.22±0.66
Sperm conc. million ml-1)	738.67a ±104.31	1138.67b ±78.82	899.0a ±87.77	1164.66b ±76.60	914.33a ±147.91	1039.67a±107.34	891.17a±95.30	925.17a ±97.57	963.92 ±38.51
Sperm abnormalities (%)									
Head	4.86a±0.61	5.33a±1.22	6.46a±0.68	5.80a±0.79	5.66a±0.80	5.36a±0.80	5.36a±0.87	5.30a±0.73	5.58±0.28
Midpiece	1.66a ±0.71	1.66a ±0.71	1.43a ±0.54	3.50b±0.67	2.00ab±0.25	2.00 ab±0.51	1.83 ab±0.40	2.32 ab±0.68	2.18±0.32
Tail	2.83 ab±0.47	2.16 ab±0.54	1.33 b±0.54	2.00 ab±0.81	2.33 ab±0.66	2.91 a±1.18	2.26 ab±0.30	1.73 ab±0.70	2.09±0.21
Total	9.35 a±0.30	9.15 a±0.74	9.22 a±3.6	11.30 a±4.62	9.99 a±1.4	10.27 a±0.98	9.45 a±1.02	9.35 a±1.80	9.85±0.23

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

Sperm Mass Activity

Average mass activity of the sperms of eight buffalo bulls (MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII) was 3.73±0.21, 3.83±0.16, 3.83±0.16, 3.5±0.22, 3.33±0.21, 3.83±0.16, 3.83±0.16 and 3.66±0.21, respectively, with an overall average of 3.64±0.07. There was no significant variation of sperm mass activity between bulls and replicates in the present study.

Progressive Sperm Motility

The mean values of progressive motility of sperm of neat semen of MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII bulls were 71.66 ± 1.05, 70.83 ± 0.83, 72.5 ± 1.11, 72.5 ± 1.11, 70.83 ± 0.83, 72.5 ± 1.11, 70.83 ± 0.83, 70.83 ± 0.83 with an overall mean of 71.56 ± 0.37 %. There was no significant variation of progressive sperm motility between bulls and ejaculates in the present study.

Live Sperm

The live sperm (Plate 10) percent in the semen of the eight Murrah buffalo bulls (MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII) averaged 83.17±2.99, 83.50±3.11, 84.66±3.11, 88±0.5, 87±1.36, 87.67±1.20, 89.00±1.00 and 84.83±1.70 % respectively, with an overall average of 86.22±0.66 %. No significant variation of live sperm percent between bulls and replicates was recorded in the present study.

Sperm Concentration

The sperm concentration in the semen of eight Murrah buffalo bulls averaged 738±104.31, 1138.66±78.82, 899±87.77, 11.64.66±76.60, 914.33±147.91, 1039.66±107.34, 891.16±95.30 and 925.16±97.57 (million ml^{-1}) for MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII bulls, respectively. The average concentration of the sperm of all the eight bulls was 963±38.51 million ml^{-1}. Sperm concentration of bull MB II and MB IV was significantly higher (P<0.05) as compared to the other bulls. No significant difference between sperm concentration of bulls MB I, MB III, MB V, MB VI, MB VII and MB VIII and bulls MB II and and MB IV was, however, recorded.

Sperm Abnormalities

The head, mid-piece, tail and total abnormalities of bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII bulls were 4.86 ± 0.61, 5.33 ± 1.22, 6.46 ± 0.68, 5.80 ± 0.79, 5.66 ± 0.80, 5.36 ± 0.80, 5.36 ± 0.87, 5.30 ± 0.73; 1.66 ± 0.71, 1.66 ± 0.71, 1.43 ± 0.54, 3.50 ± 0.67, 2.00 ± 0.25, 2.00 ± 0.51, 1.83 ± 0.40, 2.32 ± 0.68; 2.83 ± 0.47, 2.16 ± 0.54, 1.33 ± 0.54, 2.00 ± 0.81, 2.33 ± 0.66, 2.91 ± 1.18, 2.26 ± 0.30, 1.73 ± 0.70; 9.35 ± 0.30, 9.15 ± 0.74, 9.22 ± 3.6, 11.30 ± 4.62, 9.99 ± 1.40, 10.27 ± 0.98, 9.45 ± 1.02 and 9.35 ± 1.80, percent respectively, with an overall head, mid-piece, tail and total abnormalities of 5.58 ± 0.28, 2.18 ± 0.32, 2.09 ± 0.21 and 9.85 ± 0.23 percent, respectively.

No significant variation between bulls was recorded between sperm head abnormalities of the eight bulls in the present study. Bull MB IV had significantly (P<0.05) higher sperm midpiece abnormalities as compared to bulls MB I, MB II and MB III. No significant difference between sperm midpiece abnormalities between the bulls MB I, MB II, MB III, MB V, MB VI, MB VII and MB VIII and bulls MB IV, MB V, MB VI, MB VII and MB VIII was, however, recorded. Significantly higher (P<0.05) sperm tail abnormalities were recorded in the bull MB VI as compared to MB III. No significant difference was, however, observed between bulls MB I, MB II, MB III, MB IV, MB V, MB VII and MB VIII in the present study. Total sperm abnormalities, however, did not differ between bulls and ejaculates in the present study.

Cryopreserved Semen

Studies were conducted to investigate the different physico-morphological parameters of cryopreserved Murrah semen. The effect of cryopreservation on these parameters was studied by statistical comparison of values of these parameters in neat and cryopreserved semen.

Physico-morphological Parameters

The results of the investigations are summarized in Table 2-3 and the salient findings are documented as under.

Progressive Sperm Motility

The progressive motility of the cryopreserved semen of eight Murrah buffalo bulls were 53.33±1.66, 51.66±1.22, 57.5±1.11, 55.00±1.29, 53.33±1.66, 55.83±1.5, 55.00±1.29 and 50.83±0.98,percent respectively for MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII with an overall average of 54.06±0.52 percent. There was significant decline (P< 0.01) of neat semen progressive motility during the process of cryopreservation. A significant (P<0.05) variation between bulls was recorded in the present study. The post thaw progressive motility was significantly (P<0.05) higher in bull MB III as compared to bulls MB II, V and VIII. No significant difference between bulls MB III, IV, VI and VII was, however, recorded.

Live Sperm

The live sperm (%) in the neat semen of Murrah buffalo bulls MB I (85.17±2.99), MB II (83.50±3.11), MB III (84.66±3.11), MB IV (88±0.5), MB V (87.00±1.36), MB

VI (87.66±1.20), MB VII (89.00±1.00) and MB VIII (84.83±1.70) and overall average (86.22±0.66) of all the eight bulls decreased significantly (P< 0.01) after freezing and thawing (0 h) to 79.83±2.50, 72.5±6.5, 72.5±6.54, 79.16±2.27, 71.33±5.37, 77.16±2.32, 73.5±6.47and 77.00±0.89 for MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII bulls, respectively with an overall average of 76.04±1.39. Significantly (P<0.05) higher percent live sperm was recorded in bull MB I as compared to bulls MB II, III and V. No significant difference between bulls MB II, III, IV V, VI, VII and VIII was, however, recorded.

Sperm Abnormalities

The sperm abnormalities were subdivided into head, mid-piece, tail and total abnormalities. The sperm head abnormalities of cryopreserved semen of MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII bulls was 5.13±0.79, 5.50±0.96, 7.00±1.21, 5.16±1.47, 8.50±0.67, 5.33±1.22, 5.83±1.22, 5.50±0.84 and overall mean were 6.14±0.38 %, respectively. There was no significant difference in sperm head abnormalities of neat and cryopreserved semen of the experimental animals except MB V where significantly higher (P< 0.01) sperm head abnormalities in cryopreserved semen as compared to neat semen, were recorded. The midpiece abnormalities in frozen bubaline semen in the present study were 2.16±0.60, 1.73±0.71, 1.66±0.68, 3.83±0.54, 1.83±0.60, 2.00±0.44, 1.66±0.60 and 2.40±0.36 % for the bulls MB I, MB II, MB III, MB IV, MB V, MB VI, MB VII and MB VIII, respectively with an overall average of 1.81±0.19 %. There was no significant difference in sperm midpiece abnormalities of neat and cryopreserved semen of all the Murrah bulls under study.

The total sperm abnormalities in cryopreserved semen of bulls MB I, MB II, The sperm tail abnormalities in the neat semen of bulls MB I (3.13±0.75), MB II (2.33±0.49), MB III (1.46±0.68), MB IV (2.83±1.26), MB V (2.50±0.56), MB VI (3.33±0.95), MB VII (2.63±1.15) and MB VIII (1.83±0.70) their overall mean (2.14±1.63) were not significantly different from the corresponding values 2.83±0.47, 2.16±0.54, 1.33±0.54, 2.00±0.81, 2.33±0.66, 2.91±1.18, 2.26±0.30, 1.73±0.70 and their overall mean (2.09±0.21) in neat semen.

Table 2. Physico-morphological characteristics of cryopreserved Murrah semen

Bulls …… Seminal Attributes	MB I	MB II	MB III	MB IV	MB V	MB VI	MB VII	MB VIII	Overall average
Prog. Motility	53.33a ±1.66	51.66a ±1.22	57.50b ±1.11	55.00bc ±1.29	53.33ac ±1.66	55.83bc ±1.5	55.00bc ±1.29	50.83a ±0.98	54.06 ±0.52
Live %	79.83a ±2.50	72.50bc ±6.5	72.50bc ±6.54	79.16ac ±2.27	71.33bc ±5.37	77.16ac ±2.32	73.5ac ±6.47	77.00ac ±0.89	76.04 ±1.39
Head abnormalities	5.13a ±0.79	5.50a ±0.96	7.00ac ±1.21	5.16a ±1.47	8.50bc ±0.67	5.33a ±1.22	5.83a ±1.22	5.50a ±0.84	6.14 ±0.38
Midpiece abnormalities	2.16a ±0.60	1.73a ±0.71	1.66a ±0.68	3.83a ±0.54	1.83a ±0.60	2.00a ±0.44	1.66a ±0.60	2.40a ±0.36	1.81 ±0.19
Tail abnormalities	3.13a ±0.75	2.33ac ±0.49	1.46bc ±0.68	2.83a ±1.26	2.50ac ±0.56	3.33a ±0.95	2.63ac ±1.15	1.83ac ±0.70	2.14 ±1.63
Total abnormalities	10.42a ±1.35	9.56a ±1.4	10.12a ±4.40	11.82a ±2.22	12.83a ±0.76	10.66a ±0.71	10.12a ±2.15	9.73a ±0.49	10.09 ±0.42

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

MB III, MB VI, MB VII and MB VIII (10.42±1.35, 9.56±1.40, 10.12±4.40, 11.82±2.22, 12.83±0.76, 10.66±0.71, 10.12±2.15, 9.73±0.49, respectively) and their overall average (10.09±0.42) was not significantly different from their respective values of 9.35±0.30, 9.15±0.74, 9.22±3.6, 11.30±4.62, 9.99±1.40, 10.27±0.98, 9.45±1.02 and 9.35±1.80 and their overall average (9.85±0.23) in neat semen. There was significant (P<0.05) variation between bull and ejaculate in sperm head and tail abnormalities of the eight bulls in the present study. However, no such variations were recorded in sperm midpiece and total sperm abnormalities.

Table 3. Physico-morphological characteristics of neat and cryopreserved semen

Seminal Attributes	Neat Semen	Post-thaw
Prog. Motility	71.56±0.37a	54.06±0.52b
Live %	86.22±0.66a	76.04±1.39b
Head abnormalities	5.58±0.28a	6.14±0.38a
Midpiece abnormalities	2.18±0.32a	1.81±0.19a
Tail abnormalities	2.09±0.21a	2.14±1.63a
Total abnormalities	9.85±0.23a	10.09±0.42a

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

4. Discussion

Neat Semen Quality of Murrah Bulls

Semen Volume

The average semen volume of the eight bulls (2.89±0.14 ml) recorded in the present study agreed well with the findings of [40] (3.19 ml), [70] (2.78 ml) and [75] (3.30 ml) however, others have reported higher (3.60 ml: [71] 3.99 ml: [70] semen volume. These differences may be due to difference in age [76][65] genetic make up of the bull [78], season of study [80] and frequency of collection [82].

The significant variation of semen volume between bulls MB I and MB VIII and MBIII and MBVIII as observed in the present study also with significant between bull variations reported by others [74][75]. The insignificant variation between replicates indicates that the bulls behaved in a similar fashion throughout the study period as observed by [75].

Mass Activity

The mass activity of the eight Murrah bulls averaged 3.64 ± 0.07 in the present study. This was in close resemblance to the findings of [49] (3.91), [33] (3.46), [28] (3.52), [30] (3.38) and [56] (3.62). The mass activity of the semen did not vary significantly between the bulls and replicates which corroborate well with findings of [56]. The reason for no significant variation between bull and replicates may be due to the use of semen of bulls of known mass activity, same breed and identical managemental conditions and frequency of collection.

Progressive Sperm Motility

Post thaw motility of 40% and above is generally accepted for further use in artificial insemination and below it is discarded [76]. The average progressive sperm motility of the eight Murrah bulls in the present study was 71.56 ± 0.37 percent. In compliance to our study, several other workers suggested similar progressive sperm motility (73.70 %: [24] 72.92 %: Pandey, [31]; 68.32 %: [21]; 66.66 %: [40]. However, several other workers [30][34][37] recoded higher progressive sperm motility of Murrah semen (75- 80 percent), which may be due to differences in the season of study [24], age of the experimental animals [10][1] and genetic variation between the bulls [5]. There was no significant between bulls and between ejaculate variation in the progressive sperm motility of the eight bulls, which suggests that the bulls ejaculated good quality semen throughout the period of study.

Live Sperm

The mean live sperm (%) in the neat semen of the eight Murrah bulls in the present study was 86.22 ± 0.66 which complied well with the findings of [5] (87.62 ± 0.49). However, some workers reported higher (89.39 ± 0.99: [5], 88.07 ± 0.59: [8], 88.75 ± 1.44: [7] and 89.07 ± 0.44: [11] and lower values (78.80 ± 3.20: [4]; 79.72 ± 1.11: [5]; 71.44 ± 1.54: [9]. To some extent this discrepancy may be due to variations in age of the bulls and different agroclimatic conditions [16][17]. There was no significant variation between bulls and ejaculates in the present study which may be due to use of bulls of known good fertility and maintenance of identical and optimal conditions of feeding and management throughout the study period.

Sperm Concentration

The average sperm concentration of eight Murrah bulls in this study was 963.92±38.51 million spermatozoa per ml. The sperm concentration observed in the present study is in fair agreement of the findings of [3] (1060.0 million ml^{-1}), [2] (955.37 million ml^{-1}), [4] (1050.28 million ml^{-1}), [7] (1023.79 million ml^{-1}) and [9] (1046.64 million ml^{-1}). However, considerably lower (682.0 million ml^{-1}: [35][32]; 804.27 million ml^{-1}: [65] and higher (1452.00 million ml^{-1}: [55][70] 1365.15 million ml^{-1}: [51]; 1341.12 million ml^{-1}: [58]; 1335.42 million ml^{-1}: [72]; 1155 million ml^{-1}: [70] values were recorded by other researchers. The differences may be possibly due to age, [30] season [54][69] and ejaculation frequency [65]. Other workers [45][40] also opined that sperm concentration is influenced by age, ejaculation frequency, season and breed of the bulls. The significant (P< 0.05) variation of sperm concentration between bulls recorded in the present study may also be attributed to these factors.

Sperm Abnormalities

The overall total sperm abnormalities (9.85±0.23 %) in the present study are fairly comparable with the findings of [81][83][85]. Several other workers have, however, recorded higher percent total sperm abnormalities (14.25 %: [87]; 14.50 %: [88] and 11.33 %: [90]. Lower percent total abnormal sperm were, however, also recorded by few workers [56][57] No significant between bull and between ejaculate variation was recorded in the present study, which is compliance to the findings of [55].

The average head and midpiece abnormalities of spermatozoa in the present study were 5.58±0.28 and 2.18±0.32 %, respectively. Fairly comparable finding were recorded for sperm head [40][46][48] and midpiece abnormalities [80][81][83] in the past. There was no significant between bull and between replicate variations in the sperm head abnormalities in the present study. Similar findings were recorded by [31][34][36]. Significant between bull variation (P<0.05) was recorded in the midpiece abnormalities of the Murrah bulls in the present study. This was in compliance to the findings of [35]. Tail abnormalities (2.09±0.21 %) in the present study were considerably lower as compared to several other workers [22][23] which may be due to the use of bulls with known good spermiogram and ideal managemental conditions. The slight variation of the sperm abnormalities in the present study with those of the earlier reports may be due to differences in the age of bulls, season of study and frequency of collection [79][81].

Cryopreservation of Murrah Semen

Progressive Sperm Motility

The average progressive sperm motility of the eight bulls in neat semen (71.56±0.37 %) declined significantly (P< 0.01) to after freezing and thawing (54.06±0.52 %) post-thaw. The significant decline in the progressive sperm motility of semen in the present experiment is in accordance with the earlier reports (60.8 %) [23]; 53.0 %: [44]. Some researchers have also reported even lower retention of progressive sperm motility after cryopreservation of buffalo semen (48.75 %: [88]; 41.49 %: [54]; 37.88 %: [56]; 47.50 %: [22]. The difference may be due to the use of bulls of constantly good spermiogram and freezability of semen over past several years in the present experiment.

Though the eight bulls did not differ significantly in their neat semen progressive sperm motility, however, significant (P < 0.05) difference between bulls was observed in post-thaw progressive sperm motility, which is in agreement with earlier reports [38][23] indicating bull to bull variation in freezability of semen.

Live Sperm

The average live sperm (%) in the semen of eight bulls (86.22±0.66 %) declined significantly (P< 0.01) after freezing and thawing (76.04±1.39). Similar decline in live sperm (%) has also been observed in the past (8.0 %: [44]; 10.0 %:[70]. Considerably lesser decline of live sperm (%), recorded in the present study, as compared to several other researchers (14.0 %: [86]; 50.0 %: [19]; 33.0 %:[90] may be due to use of bulls of known good spermiogram and freezability. Significant (P<0.05) between bull and ejaculate differences of percent live sperm in cryopreserved semen recorded in the present study complied well to the findings of [3][4]. Though there was no significant (P < 0.05) differences between the percent live sperm of the eight bull, presence of such differences at post thaw stage points towards differences in freezability of the ejaculates.

Sperm Abnormalities

The average total sperm abnormalities of neat semen (9.85±0.23 %) in the present study were significantly different from those observed after freezing and thawing (10.09±0.42 %) post-thaw. Comparable values of post thaw total sperm abnormalities were also recorded in the past [1][3]. No significant difference observed between neat semen post thaw total sperm abnormalities point to the use of bulls with known good spermiogram and excellent freezability. This may also be due to use of strict care and adherence to the standard protocol for freezing and thawing which might have caused lesser induction of abnormalities due to thermal shock or osmotic damage during the process of cryopreservation.

Correlation of Sperm Morphometry Parameters with Physico-morphological Characteristics

The correlation study between sperm morphometry parameters and physic-morphological characteristics of neat semen of Murrah bulls revealed significant negative correlation between sperm concentration and sperm head length (r= - 0.5260), sperm head width (r= - 6502), width at base (r= -6459), tail length (r= -5503) and total sperm length (r= -0.6850) and significant positive correlation with ellipticity (r=0.4970), head area (r=0.3080), head shape (0.4509) and elongation (r= 0.4233). There was a significant positive correlation of sperm mass activity with sperm head length (r= 0.6210), mid piece length (r= 0.7646), tail length (r=0.4361), sperm head shape (r=0.6069), Ellipticity (r= 0.3962), head area (r= 0.4778). Progressive sperm motility was also significantly positively correlated with sperm head length (r= 0.525), mid piece length (r= 0.648), tail length (r= 0.5631), total length (r= 0.7696), sperm head area (r= 0.6049) and head shape (r= 0.6399) and significantly negatively correlated with ellipticity (r= - 0.3563). A significant positive correlation of percent live sperm with sperm head length (r= 0.644), head width (r= 3930), midpiece length (r= 0.6140), tail length (r= 0.3387), total sperm length (r= 0.3996), sperm head area (r= 0.5289) and head shape (r= 0.5832) and significant negative correlation with ellipticity

(r= - 0.3062) was also recorded in the present study.

The sperm head abnormalities in neat semen of Murrah bulls in the present study were significantly positively correlated with sperm head length (r=0.5340), head width (r=0.6069), head area (r=0.7640), head shape (r=0.5780), width at base (r= 0.5262) and midpiece length (r= 0.5582). No significant correlation of sperm head abnormalities with any other sperm morphometry parameters was, however, recorded in the present study. The sperm midpiece abnormalities were significantly positively correlated with sperm head length (r=0.6175). A significant negative correlation of midpiece abnormalities with head width (r= - 0.3082), midpiece length (r= - 0.6278), total sperm length (r= - 0.3610), head shape (r= - 0.5951) and elongation (r= -0.6452) was also recorded in the present study. There was significantly positive correlation sperm tail abnormalities with width at base (r=0.6044), and ellipticity (r=0.666) and significant negative correlation with head width (r= -0.776), midpiece length (r= - 0.5162), tail length (r= - 3076), elongation (r= - 3616) and sperm head area (r = - 0.7722). In the present study, the total sperm abnormalities were significantly negatively correlated with midpiece length (r= - 0.5442), tail length (r= - 0.4509), total sperm length (r = - 0.3775), head area (r = - 0.3237), head shape (r= - 4920), ellipticity (r= -0.7144) and elongation (r= -0.6272) and significantly positively correlated with the sperm head length (r= 0.711). Similar trends were observed in case of correlation of sperm morphometric parameters with physicomorphological characteristics in cryopreserved semen also (table 1and 2).

5. Conclusions

In the present study, there was significant between bull and between ejaculate variation in progressive sperm motility, live %, sperm head tail abnormalities.

A significant decline of the progressive sperm motility and live sperm % was recorded in cryopreserved semen as compare to the neat semen whereas no significant difference between the sperm abnormalities was recorded between neat and cryopreserved semen.

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