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Research Article

MORPHOLOGY AND FERTILITY STATUS OF SPERMS IN FROZEN MURRAH BUFFALO BULL SEMEN UPON STORAGE

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ABSTRACT

The present investigation was undertaken to study the sperm morphology in relation to fertility of frozen semen of Murrah bulls stored for a period of seven months at an organized animal farm in liquid nitrogen can. The semen straws from each bull (N=8) were thawed every month at 37°C for 30 seconds and evaluated for motility, sperm abnormalities, per cent live and dead spermatozoa and per cent intact acrosomes. A total of 126 buffaloes of 2nd to 4th lactation were inseminated with frozen-thawed semen of eight Murrah buffalo bulls during the entire period of investigation, with a total of 12-25 inseminations per bull. Pregnancy diagnosis was done by per-rectal examination after 60-90 days and correlation ($P<0.05$) of conception rate with progressive sperm motility and live spermatozoa of Murrah buffalo bulls semen was carried out. Significant ($P<0.05$) positive correlations were obtained between progressive sperm motility, live spermatozoa and conception rate. Thus, it can be concluded that seminal parameters play an important role in determining the fertility of Murrah buffalo bull semen as well as the semen quality is affected by storage under farm conditions due to regular handling.

Keywords: Livability, Murrah buffalo bulls, Motility, Semen, Sperm abnormalities

Semen cryopreservation and artificial insemination (AI) offer many advantages to the livestock industry, particularly in conjunction with genetic evaluation and selection programs. Semen cryopreservation has been widely used for preserving semen of farm animals, including buffaloes (Sansone *et al.,* 2000) but the fertility of frozen semen in buffaloes remains poor as compared to fresh semen and is recorded to be 33.00 percent (Chohan *et al.,* 2006). The biggest obstacle in making use of cryopreserved semen of many species is that freezing and thawing generally damage sperm membrane structures, leading to fewer viable and motile cells post-thaw (Hammerstedt *et al.*, 1990). Artificial Insemination (AI) is one of the most successful reproductive technologies developed for rapid disposal of superior germplasm to improve productivity of dairy animals and preventing dissemination of venereal diseases (Bols *et al.*, 2010). Success of AI depends upon the regular and continuous availability of good quality semen. Although, AI has been practiced for past 50 years, fertility rate with this technology is less and unpredictable in buffaloes (Agarwal and Tomer, 2003). Visual assessment of the proportion of motile spermatozoa is the most commonly used viability test to predict fertility. Thus, fertility is the only reliable and accurate determinant of quality of frozen semen in various species of domestic animals including buffaloes.

The present investigation was, therefore planned with the objective to study various semen characteristics of same batch of frozen semen of Murrah buffalo bulls stored for a longer period at animal farm at monthly intervals as well as to find out correlation between conception rate and seminal parameters of frozen semen.

MATERIALS AND METHODS

Frozen semen of eight Murrah buffalo bulls was procured and analyzed for various morphological characteristics of sperm, viz, progressive sperm motility, live and dead spermatozoa, abnormal spermatozoa and percent intact acrosome. The semen samples were evaluated at monthly interval for a period of seven months in the same liquid nitrogen can from which the semen straws were being used for AI of buffaloes at the farm.

Two mini straws (0.25 ml) of frozen semen from each bull were thawed at 37 °C for 30 seconds in water bath. Immediately after thawing, semen was transferred to eppendorf tubes and kept at 37 °C in a water bath. The progressive sperm motility of semen of each bull was recorded by placing a small drop of thawed semen on a pre-warmed (37 °C) clean grease free glass slide under a cover slip and examined under high power magnification (40 X) lens. Percent live and dead spermatozoa as well as sperm abnormalities were determined using Eosin-Nigrosin stain (Campbell *et al.,* 1960). Intact and damaged acrosomes were estimated by making use of Giemsa-stain (Watson and Martin, 1972) with slight modification that the

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staining time was kept as 1 hour for buffalo bull semen. The degree of change in acrosome was estimated using phase contrast microscope under oil immersion (100 X) lens.

A total of 126 buffaloes were inseminated with frozen-thawed semen of eight Murrah buffalo bulls during the winter months of the year from August to February. A minimum of 12 and a maximum of 25 buffaloes were inseminated with frozen semen of different bulls during the entire period of investigation. Pregnancy was confirmed by rectal palpation of buffaloes 60-90 days after AI.

Statistical analysis: The data so obtained was analyzed statistically by Duncan's Multiple Range Test using SPSS/PC student-ware computer software (Norusis, 1988).

RESULTS AND DISCUSSION

The percent progressive sperm motility (Mean±SE) in frozen-thawed semen of Murrah buffalo bulls recorded bull-wise is presented in Table 1. The average progressive sperm motility ranged from 26.42±1.79 to 40.71±2.02 per cent with an overall average value of 32.50±0.93 per cent. Significant differences ($P<0.05$) among bulls were observed in progressive sperm motility. The average progressive sperm motility ranged between 25.62±1.75 to 39.37±2.39 per cent during different months (Table 2). Progressive sperm motility started declining from month of September onwards and reached to a minimum value (25.62±1.75 per cent) in the month of February. Progressive sperm motility in the present study is in agreement with the findings of Kataria and Tuli (1992) and Bhandari *et al*. (1982) who obtained 30.00 to 40.00 and 39.66 to 44.33 per cent motility, respectively, but lower as compared to the findings of Lambrechts *et al.* (1999) who obtained higher values of 56.30 per cent.

The average live sperms of each bull recorded for seven months ranged from 48.14±1.76 to 59.14±3.85 per cent with an overall average value of 53.66±1.08 per cent (Table 1). There is significant ($P<0.05$) variation in per cent live sperms among the bulls. However, there is significant ($P<0.05$) decrease in per cent live sperms in the months of August to February. Live spermatozoa reported in this investigation are in accordance with the results of Panghal and Tuli (1999) and Singh and Raina (2000) who obtained 52.00 and 49.00 per cent live spermatozoa, respectively. Lower values of 38.82 per cent live sperms were observed by Shetti *et al*. (1978).

The average abnormal sperms ranged from 5.14±0.85 to 13.57±2.29 per cent with an overall average value of 7.66±0.51 per cent (Table 1). There is significant ($P<0.05$) variation in percent abnormal sperms among the bulls. Number of abnormal sperms showed significant ($P<0.05$) increase from 5.50±1.30 to 9.37±0.92 per cent in the months of August to December and thereafter it remained constant (Table 2). Abnormal spermatozoa obtained in this investigation is in accordance with the findings of Gupta *et al.* (1978) who reported 10.50 to 13.70 per cent abnormal spermatozoa, but not in accordance with the findings of Bhosrekar (1981) who observed 17.32 to 28.77 per cent abnormal spermatozoa.

The average intact acrosomes ranged from 61.57 ± 1.78 to 80.00 ± 3.30 per cent with an overall average value of 73.73 ± 1.33 per cent (Table 1). Significant differences ($P<0.05$) among the bulls were observed. There is significant ($P<0.05$) decrease in per cent intact acrosomes starting from month of August to February (Table 2). Spermatozoa with intact acrosomes in this study are in agreement with the findings of Bandopadhyay *et al.* (1983) and Kataria and Tuli (1992) who observed 61.40 and 69.70 per cent intact acrosomes, respectively, but lower values of 48.27 per cent have been reported by Goyal *et al.* (1996). Bhosrekar *et al*. (1994) obtained higher value of 89.29 per cent intact acrosomes in frozen-thawed semen of buffalo bulls. The variation in seminal parameters of frozen-thawed semen of Murrah buffalo bulls reported in the present study might be due to the shortcomings in maintaining the frozen semen straws for storage at the farm, since the straws are stored in the same liquid nitrogen can from which semen is used daily for AI of farm animals.

The average conception rate ranged from 20.00 to 50.00 per cent with an overall average value for all the eight bulls as 26.98 per cent (Table 1). There is significant variation in conception rate among bulls. There is significant variation in conception rate from the month of August to February (Table 2). Conception rates in the present study is in accordance with the findings of Tuli *et al.* (1981) and Chohan *et al.* (2006) who obtained 35.22 and 33.00 per cent conception rates, respectively, but not in accordance with the findings of Dhami and Sahni (1994) and El-Amrawi (1997) who obtained 68.10 and 64.50 per cent conception rates, respectively. Higher conception rates reported by these researchers might be due to better semen quality of bulls used in their studies.

Table 1

Seminal parameters (Mean ± SE) and conception rate of frozen-thawed semen of Murrah buffalo bulls (bull-wise)

Bull no.	N	Progressive motility (%)	Live sperms (%)	Abnormal sperms (%)	Intact acrosomes (%)	Conception Rate (%)
1.	7	32.14^{b}±1.48	57.14^{ab}±3.29	13.57^{a}±2.29	61.57^{c}±1.78	20.00
2.	7	40.71^{a}±2.02	54.71^{bc}±2.39	6.57^{c}±0.48	73.00^{b}±1.55	50.00
3.	7	26.42^{c}±1.79	52.00^{d}±2.96	5.14^{c}±0.85	79.28^{a}±3.42	23.08
4.	7	32.14^{b}±2.14	53.28^{cd}±2.57	9.85^{b}±1.20	78.71^{a}±3.76	28.57
5.	7	38.57^{a} ±2.36	48.14^{e}±1.76	6.28^{c}±1.30	62.28^{c}±1.32	25.00
6.	7	32.14^{b}± 1.01	53.28^{cd}±3.50	7.57^{bc}±0.48	78.14^{ab}±3.04	50.00
7.	7	28.57^{c}±2.82	51.57^{d}±3.17	5.42^{c}± 0.48	80.00^{a}±3.30	23.08
8.	7	29.28^{bc}±2.76	59.14^{a}±3.85	6.85^{c}±0.82	76.85^{ab}±3.07	30.76
Overall average		32.50±0.93	53.66±1.08	7.66±0.51	73.73±1.33	26.98

N = Total no. of observations (at monthly intervals); Means with different superscripts in a column differ significantly (P< 0.05)

Table 2

Seminal parameters (Mean ± SE) and conception rate of frozen-thawed semen of Murrah buffalo bulls (month-wise)

Bull no.	N	Progressive motility (%)	Live sperms (%)	Abnormal sperms (%)	Intact acrosomes (%)	Conception Rate (%)
August	8	39.37^{a}±2.39	63.62^{a}±1.70	5.50^{a}±1.30	82.25^{a}±5.06	42.85
September	8	35.62^{b}±1.75	59.00^{b}±1.93	6.50^{ab}±1.64	79.12^{ab}±4.26	25.00
October	8	35.00^{b}±1.89	57.12^{bc}±1.54	7.25^{a}±2.08	76.37^{bc}±3.04	50.00
November	8	33.75^{b}±1.56	55.25^{c}±1.41	8.37^{ab}±1.29	73.25^{cd}±2.84	100.00
December	8	30.00^{c}±1.89	52.62^{d}±1.34	9.37^{bc}±0.92	69.87^{de}±2.14	26.31
January	8	28.12^{cd}±2.66	47.00^{e}±0.90	9.12^{bc}±0.71	68.12^{e}±1.04	6.06
February	8	25.62^{d}± 1.75	41.00^{f}±1.01	9.50^{c}±0.82	67.12^{e}±1.52	12.50

n = No. of semen samples evaluated; Means with different superscripts in a column differ significantly (P<0.05)

Table 3

Correlation coefficients between seminal parameters and conception rate

Seminal parameters	Progressive Motility	Intact Acrosomes	Live Sperms	Abnormal sperms	Conception rate
Progressive motility	–	0.080^{NS}	0.502**	0.293*	0.305*
Intact Acrosome	–	–	0.477**	0.111^{NS}	0.151^{NS}
Live Sperms	–	–	–	0.456**	0.285*
Abnormal Sperms	–	–	–	–	0.059^{NS}

NS-non significant,*-(P<0.05),**-(P<0.01)

Correlations between seminal parameters and conception rate (fertility) of frozen-thawed semen are presented in Table 3. Progressive sperm motility and live spermatozoa were highly positively (P<0.05) correlated with conception rate. There was non-significant (P>0.05) correlation between intact acrosomes and conception rate. The correlation between progressive sperm motility and live spermatozoa as well as between intact acrosomes and live spermatozoa was highly significant (P<0.01).

Different seminal parameters showed positive correlation with the conception rate, but did not reach to the level of significance which might be due to the low number of semen samples as well as less number of buffaloes inseminated per bull. Significant decrease (P<0.05) in progressive sperm motility, live spermatozoa and intact acrosomes and increase in sperm abnormalities was noticed during storage of frozen semen. There was an unusual significant positive correlation of per cent abnormal sperm with per cent live sperm and progressive sperm motility as well as non-significant correlation with conception rate. Usually, higher numbers of abnormally shaped sperm are associated with other irregularities of the semen such as low sperm count or motility. Males with abnormally shaped sperm may also have no trouble causing

a pregnancy. Having reviewed the literature, it seems clear that sperm morphology is an important parameter in the fertilization process *in vivo* and *in vitro* as well as the progressive motility of spermatozoa. It can be used as a single and independent predictor for successful fertilization. However, in the cases of *in-vivo* fertilization, the total number of available spermatozoa as well as other seminal parameters play important role in predicting the fertility (Nikolettos *et al*., 1999).

The results of present investigation and corroborated references suggest that seminal parameters definitely play an important role in determining the fertility of Murrah buffalo bull semen. However it is desirable to strategize the managemental practices at farm and use separate liquid nitrogen storage can for long time storage of same batch of semen samples.

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