Indian Journal of Animal Sciences **85** (4): 376–378, April 2015/Short communication

Age at attainment of semen freezability vis-a-vis semen characteristics and fertility in Murrah buffalo bulls

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Received: 9 October 2014; Accepted: 5 November 2014

Key words: Age at puberty, Buffalo semen, Cryopreservation, Fertility, Freezability

The semen production commences in buffalo bulls at an age less than 2 years but it requires sufficient time for improving the quality of semen from the onset of puberty till sexual maturity and quality improves gradually with the advancement of age to attain the semen freezability. The first semen donation in artificial vagina was taken as criterion to signify the puberty in young bulls (Kotaya *et al.* 1972, Bhavsar *et al*.1974) and the age at which semen characteristics are comparable to normal semen of adult bulls is taken as age at maturity (Rao and Rao 1978). An interval of 11–12 months was observed for improving the quality of semen from puberty in Ongole bulls (Rao *et al*. 2000). Kanchan *et al*. (2007) revealed that the overall mean time period acquired to achieve freezability in buffalo bulls was 5–6 months and the time period acquired to achieve semen freezability in bulls commencing semen production at age less than 2 years was significantly higher as compared to bulls commencing semen production at the age of 2.5 – 2.75 and > 2.75 years. Therefore the present study was planned to find the optimum age of young buffalo bulls for their selection in breeding programme to achieve better semen quality, freezability and fertility for production of genetically superior sires for genetic improvement of buffaloes.

Buffalo bulls (27) maintained at GADVASU dairy farm were divided into 4 age groups, viz. <24 (group 1); 24–30 (group 2); 30–33 (group 3) and >33 (group 4) months respectively based upon their age at puberty (i.e. first semen ejaculate donation). Semen was collected twice a week and the age of the bull at first successful freezing of semen ejaculate was recorded. Semen collections (4,580) were made using standard artificial vagina (AV) method and each semen ejaculate was examined for its volume (ml), consistency (graded as 0–3), mass activity (scored as 0–4), sperm concentration (millions/ml), initial motility (%), pre-freeze motility (%), post thaw motility (%). Semen was frozen in French-medium straws (20 million motile spermatozoa per straw) using tris as extender. Frozen semen with post thaw motility more than 40% was used for AI and the percent conception rate was recorded. The data were analyzed statistically by Duncan's multiple range test (one way analysis of variance) using SAS 9.3 to compare mean values of various parameters in different age groups (Snedecor and Cochran 1967).

Our result revealed that relatively higher percentage of bulls (44.50%) started semen donation at the age of 24–30 months and the average age of bull at puberty is 29.48 ± 1.07 months (Table 1), which is same as reported earlier (Kanchan *et al*. 2007) whereas average age at puberty reported earlier by others workers is 26.36 ± 1.48 months (Kotaya *et al*. 1972) and 36.85 months (Mukhopadhyay *et al*. 2010) in Murrah buffalo bulls, 23.13 months in Surti buffalo bulls (Bhavsar *et al*.1974) and 25 months in Nili Ravi buffalo bulls (Ahmad *et al*.1984). The difference in the results on the age at puberty might be due to the difference in breed, management and number of bulls studied.

The mean age of bull at first successful frozen semen production was 33.93 ± 1.00 months in the present investigation. Similar results were also reported earlier (Kanchan *et al*. 2007). However Mukhopadhyay *et al*. (2010) observed an average age at first freezing to be 40.18 months in Murrah buffalo bulls. The result showed that the average age at first successful semen ejaculate freezing was significantly higher ($P \leq 0.05$) in the fourth age group bulls (i.e.>33 months) as compared to other age group bulls (Table 1). The results showed that although buffalo bulls started donating semen at an age less than 2 years but requires sufficient time to attain the semen freezability. These results commensurate with the previous findings (Kanchan *et al*.2007). During this period various biochemical, physiological and morphological changes occur in semen to withstand the shock of cryopreservation. The overall time period acquired to achieve freezability in buffalo bulls was 4.45 months. However, earlier it was reported to be 5.7 months and this variation in results may be due to difference in the number of bulls, total number of ejaculates undertaken and also due to individual variation (Kanchan *et al.* 2007).

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The mean value of various parameters viz., semen volume, consistency, sperm concentration, mass activity, average sperm motility, prefreeze motility, post thaw motility, percent ejaculate cryopreserved and conception rate are given in Table 1. The average semen volume observed in present study was similar to that reported in previous study (Kanchan and Singh 2005), however it does not commensurate with the previous findings by others (Kumar *et al*.1993, Bhosrekar *et al*. 1994, Sahu and Pandit 1997, Pratap *et al*. 1999, Javed *et al.* 2000, Bhakat *et al*. 2011) Variation in semen volume reported by different workers might be due to difference in genetics, reproductive health status of bulls, age of bulls, frequency of collection, pooled volume, nutrition, season and management. Variations can also be due to skill of semen collector / attendant and temperature of AV.

The average sperm concentration was 724.74 ± 131.67 million/ml which is not in agreement with previous reports by others in Murrah buffalo bulls, viz. 849.32 ± 22.64 millions/ml (Kumar *et al*. 1993); 2.04 ± 0.04 × 10^9 sperm / ml (Bhosrekar *et al*. 1994); 1311.18 ± 38.36 millions/ml (Sahu and Pandit 1997); 1053.28 ± 91.93 millions/ml (Pratap *et al*. 1999); 998± 10.90 (Bhakat *et al*. 2011). Such variation can always be expected from workers working at different places with a variation in the number of animals selected belonging to different age groups.

The average mass activity (2.72 ± 0.09) observed during the present study commensurate with previous findings (Pratap *et al*.1999, Javed *et al*. 2000). The average sperm motility (%) was 67.91 ± 0.71 (Table 1) which is similar to as reported earlier (Matharoo *et al*.2007, Pratap *et al*.1999, Bhakat *et al.* 2011). However it is different as reported by others (Kumar *et al*.1993, Sahu and Pandit 1997, Javed *et al*. 2000). The difference in motility in various reports could be due to the variation in judgment of motility, number of bulls studied or due to different seasons. The average prefreeze motility observed in present studies is almost same as reported earlier (Matharoo *et al*.2007, Rao *et al.*2002). The average post thaw motility was same as that found earlier in our previous studies (Matharoo *et al*. 2007, Kanchan and Singh 2005). However the results are not in agreement as reported by others (Sahu and Pandit 1977, Rao *et al*. 2002). The results showed that the post thaw motility of semen in fourth age group bulls was significantly higher (P≤0.05) as compared to post thaw motility observed in bulls belonging to other age groups (Table 1) and thereby, results suggested that freezability of semen improves gradually with the advancement in age of bulls.

The average per cent conception rate in present studies was 29.69 ± 1.36 (Table 1) which is same as reported earlier by Anzar *et al*. (2003) and Kanchan and Singh (2005). However it was 40.30 ± 2.70 and 34.12 ± 2.59 % in Murrah buffalo bulls (Rao *et al*. 2002, Bhosrekar *et al*. 2003) and varied from 40–60 % in buffalo bulls (Saji 1984). In the present study the conception rate did not vary significantly among the bulls of 4 age groups despite the fact that post thaw motility was significantly high in the fourth age group (i.e. >33 months) as compared to other age groups. However, in previous studies, a highly significant positive correlation was indicated between fertility and percentage of the post thaw motility of spermatozoa (Gibson and Graham 1969, Chandler *et al*. 1978). The minimum limit of motility after thawing for sperm of normal fertility is 20% as reported by Elliot (1976) and the minimum limit of post thaw motility in the present studies is 40% in all the age groups of bulls and probably this is the reason for no variation in percent of conception rate in 4 different age groups. The conception rate seems to be dependent upon the semen quality, individual variation in freezability, efficiency of processing, storage and handling the frozen semen rather than the age of bull itself, it is dependent upon several other factors, viz. breed, body condition, heat sign, nutrition, management and also upon the skill of AI technician as already known.

From the results it is suggested that the breeding bulls should be selected at an appropriate age of sexual maturity i.e. >33 months for inclusion in breeding programme to meet the frozen semen production targets. It is also

Table 1. Age at attainment of semen freezability, semen characteristics and conception rate in Murrah buffalo bulls

Parameters	Age groups (months)				
	<24 Group 1 (n=4)	24–30 Group 2 (n=12)	30–33 Group 3 (n = 5)	>33 Group 4 (n=6)	Mean ± SE (n = 27)
Age at first semen collection (months)	22.00^a ± 0.41	27.17^b ± 0.56	31.80^c ± 0.37	37.17^d± 1.67	29.48± 1.07
Age at first semen freezing (months)	33.75^a ± 1.84	30.92^a ± 0.97	33.40^a ± 1.12	40.50^b ± 1.12	33.93 ± 1.00
Average ejaculate volume (ml)	3.35^a ± 0.29	3.60^a ± 0.34	3.27^a ± 0.79	3.29^a ± 0.23	3.43± 0.20
Consistency (graded as 0–3)	2.41^a ± 0.21	2.56^a ± 0.08	2.53^a ±0.19	2.66^a± 0.06	2.55 ± 0.06
Sperm concentration (millions/ml)	584.25^a ±338.47	553.50^a±202.42	980.00^a±264.42	948.17^a ± 309.36	724.74 ±131.67
Mass activity (scored as 0–4)	2.53^a ± 0.16	2.79^a± 0.14	2.49^a± 0.24	2.90^a ± 0.20	2.72 ± 0.09
Average sperm motility (%)	65.50^a ± 1.55	67.50^a ± 0.86	68.40^a± 2.25	69.92^a ± 1.47	67.91 ± 0.70
Pre-freeze motility (%)	59.75^a ± 1.84	62.00^a ± 1.13	61.40^a ± 1.32	63.50^a ± 1.11	61.89± 0.67
Post thaw motility (%)	39.66^a ± 1.70	41.78^a ± 1.12	39.30^a ± 2.18	45.02^b ±0.96	41.72 ± 0.78
Conception rate (%)	28.29^a± 2.50	29.40^a± 2.15	32.48^a ± 4.51	28.86^a ± 2.30	29.69 ± 1.36
Ejaculate cryopreserved (%)	44.78^a ± 2.78	46.82^a ± 3.90	39.96^a ± 7.91	46.29^a ± 3.91	45.13 ± 2.38

Mean values with similar superscripts did not differ significantly and means with dissimilar superscripts differ significantly at P≤0.05.

suggested that newer approaches of semen analysis using computer assisted semen analyzer (CASA) and Easy-cyte flow cytometer should be incorporated along with routine conventional semen evaluation to achieve high conception rate of buffalo bulls.

SUMMARY

The present study was planned to find the optimum age of young buffalo bulls for their selection in breeding programme to achieve better semen quality to fulfill frozen production targets and to achieve high fertility for production of genetically superior sires. Buffalo bulls (27) were divided into 4 age groups viz. <24,24–30, 30–33 and >33 months, respectively, based upon their age at puberty. Semen was frozen in French medium straws using Tris as extender. Various parameters were studied for each bull and their mean values were compared in different age groups. The results revealed that the overall age of bull at puberty and first successful frozen semen production was 29.48±1.07 and 33.93±1.00 months, respectively. The results showed that the age at first successful semen ejaculate freezing was significantly higher ($P \leq 0.05$) in fourth age group bulls as compared to other age group bulls. The post thaw motility of semen in fourth age group bulls was significantly higher as compared to post thaw motility of other age group bulls. However conception rate did not vary significantly among the bulls of 4 age groups. It is suggested that the breeding bulls should be selected at an appropriate age of sexual maturity i.e. >33 months of their age for their inclusion in the breeding programme to meet the frozen semen production targets.

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