STUDIES ON SEMINAL ATTRIBUTES IN RELATION TO CHROMOSOMAL PROFILE IN MURRAH BUFFALO BULLS

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ABSTRACT

Seminal attributes and karyological study was conducted on twenty five Murrah buffalo breeding bulls belonging to Central Semen Station, Bhopal. Five semen samples from each buffalo bull were collected at 7 days interval. Fresh semen was evaluated for volume, mass motility, sperm concentration, progressive motility, live sperm count and morphological abnormalities. The mean volume, mass motility, progressive motility, sperm concentration, live sperm count and total abnormal sperms were 2.71±0.112%, 3.34±0.073%, 68.4±1.30%, 978.9±34.37%, 89.52±0.38%, and 8.82±0.873%. Significant ($P<0.05$) variation was observed between the bulls with regard to sperm abnormalities. However, non-significant difference was observed for volume, sperm concentration, mass motility, progressive motility, and live sperm count. The overall mean sperm abnormalities found for abnormal head, middle piece and tail were 1.68±0.160%, 2.0±0.238%, and 5.12±0.475%, respectively. Tail abnormalities differed significantly between bulls. Karyological screening of conventionally stained slides and G and C banded slides of breeding bulls did not reveal any deviation from normal diploid chromosome number and morphology of water buffaloes. The presence of structural abnormalities like gaps and breaks were observed at very low frequency. In general the centromeric region was G-band negative. The centromeric hetrochromatin of submetacentric autosomes stained faintly than that of acrocentric autosomes, the X-Chromosome had a prominent C-band.

Keyword: *Bubalus bubalis*, buffaloes, karyological, progressive mortility, centromeric region

INTRODUCTION

Selection of young bulls at an early age is crucial for commercial semen producers (Hafez and Hafez, 2000). A deficiency in bull reproductive traits has a larger impact on herd productivity

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and fertility problems than in a single female (Rodriguez-Martinez, 2008). Evaluation of semen quality has been based on routine semen analysis (motility, morphology and acrosome integrity, Selvaraju *et al*., 2008). Chromosomal abnormalities account for a substantial loss in animal production and problem related to fertility which can be screened even at calf hood stage and the abnormal animal may be removed from breeding programme to avoid transmission of such abnormalities to their progenies. About 8% of reproductive deficient animals have shown chromosomal abnormalities and hence timely culling of such bulls will improve fertility of the herd (Pattanayak *et al*., 2007).

MATERIALS AND METHODS

The present study was conducted on Twenty five Murrah buffalo breeding bulls belonging to Central Semen Station, Bhopal. Semen ejaculates were harvested from bulls using Swedish pattern, artificial vagina. From each bull five samples were collected, at an interval of 7 days. Semen evaluation was done immediately after collection. Volume of the semen was noted directly from the graduations of semen collection glass tube. Mass motility was observed as per the procedure described by Herman and Madden (1953). The individual motility of spermatozoa was expressed in terms of percentage of progressively motile spermatozoa (Zemjanis, 1970). Spermatozoa concentration (million/ml) was determined using photo colorimeter (IMV), standardized at 530 n (Willet and Buckner, 1951). Vital count of spermatozoa (percentage) was estimated using Eosin-Nigrosin staining technique (Campbell *et al*., 1956). Sperm morphological abnormalities in head, middle piece and tail were studied as per the procedure described by Rao (1971). For karyological studies short term lymphocyte culture technique using RPMI 1640 culture medium was adopted (Moorhead *et al.*, 1960). Pokeweed was used as mitogen. G and C banding were done by standard procedure. A total of 20 to 30 metaphase plates were screened for each bull. General morphology and number of chromosomes along with abnormalities in morphology and numbers if any was recorded.

RESULT AND DISCUSSION

Seminal attributes volume

The overall mean semen volume recorded was 2.71±0.112 ml (Table 1) ranging from 2±0.332 to 4.4±0.87 ml. Analysis of variance revealed non-

Table 1. Seminal attributes of buffalo breeding bulls (mean±SE).

Seminal attributes	Mean±SE	Morphological abnormalities	Mean±SE (%)
Volume(ml)	2.71±0.11	Head abnormalities	1.68±0.16
Sperm concentration (mill/ml)	978.9±34.37	Mid piece abnormalities	2.0±0.23
Mass activity(score)	3.34±0.07	Tail abnormalities	5.12±0.47
Progressive motility (%)	68.4±1.30	Total abnormalities	8.82±0.87
Live sperm count (%)	89.52±0.38		
Sperm Morphology (%)	11.12±0.41		

significant difference (P<0.05) in the volume of semen between bulls. This is in agreement with the findings of Tiwari *et al.* (2009) who reported almost similar volume of semen in Tarai and Surti buffalo bulls. Patel *et al.* (2012) reported higher values in Jafarabadi, Mehsana and crossbred (HF x Kankrej, F1). These variations may be attributed to breed variations.

Mass motility

Based on 0 to 5 scale, the mean mass motility (3.34±0.073) of spermatozoa in the semen of breeding bull ranged from 2.6 to 4.0 (P>0.05). These observations are in close resemblance to the findings of previous workers (Shukla and Misra, 2005).

Progressive motility

The overall mean progressive motility of spermatozoa in present study was found to be 68.4±1.30%, ranging between 55 to 77% Between bull variation in the progressive motility of spermatozoa was found to be statistically non significant. The present findings are in close agreement with the observations of Pandey (2001); Narayan *et al*. (1999), though, Selvaraju *et al.* (2008) reported lower progressive motility in Murrah bulls.

Sperm concentration

The mean value of sperm concentration (978.9±34.37 million/ml) in semen of buffalo breeding bulls, ranged from 632.8 to 1335.8 million/ml. The present results corroborate with the findings of Tiwari *et al.* (2009) in Tarai buffalo bulls. Selvaraju *et al*. (2008) reported a very higher concentration of sperm in Murrah buffalo bulls.

Live sperm count

The overall mean live spermatozoa count was 89.52±0.38%, with a range of 86.4 to 92.6%. Statistically it did not reveal significant difference between the bulls. The present study is in agreement with the finding of Shukla (2002), who also recorded similar mean percent live spermatozoa in the neat semen of the Murrah bulls.

Sperm abnormalities

The mean percentage of abnormal spermatozoa in the semen of different bulls was 8.82±0.873%, which is fairly well comparable with the findings of Pandey (2001); Patel (2011). Some other workers reported higher percent of total sperm abnormalities (Nath *et al*., 1991; Shabd, 1998). A few workers reported lower percent total abnormal sperm abnormalities (Singh *et al*., 2000; Perumal *et al*., 2009) however, Shukla and Misra (2005) observed statistically non-significant difference between bulls. The average head and mid-piece abnormalities of spermatozoa in the present study were 1.68±0.160 and 1.71±0.285 respectively. Fairly well comparable finding were recorded for sperm head (Gunarajsingham *et al*., 1996, Veerabramhaiah *et al*., 2010; Patel, 2011) and for mid-piece abnormalities (Shukla, 2002; Patel, 2011). There was no significant variation between bull and between replicate variations in the sperm head abnormalities in the present study. Similar findings were recorded by Shukla and Misra (2005); Siddiquee *et al.* (2011); Patel (2011). Significant between bull variation (P<0.05) was recorded in the mid-piece abnormalities of the Murrah bulls in the present study. This was in compliance to the findings of Shukla and Misra (2005). As regards tail abnormalities (2.09±0.21%) in the present study are concerned they were considerably low as compared to several other workers (Gunarajsingam

et al., 1996; Patel, 2011).

Cytogenetic studies

In the present study the observed diploid chromosomes number was 2n=50. The karyotype comprised of 5 pairs of submetacentric, 19 pairs of acrocentric autosomes and 1 pairs of acrocentric sex chromosomes. The observed G-band and C-band patterns were in closed agreement with the features reported by earlier workers in river type buffalo (Bongso and Hilmi, 1982; Thiagrajan, 1987; Yadav, 1981; Yadav and Balkrishan, 1982). In the present study structural abnormalities were recorded in the form of gaps and breaks at very low frequency. This might be associated with mechanical losses during harvesting and preparation of slides. Many of the chromosomal abnormalities in cattle breeding bulls have been reported by various workers (Kieffer and Cartwright, 1968; Mandal *et al*., 2003; Ahmad *et al.*, 2004). However, availability of such reports in buffalo breeding bulls is very scanty. In the present study no abnormality could be traced out in any of the breeding bulls. This could be due to the fact that all the bulls maintained at central semen station have been procured from selected stock and also the buffaloes have less prominence and aesthetic values compared to sacred cattle consistently they are slaughtered more frequently with any physical or reproductive abnormalities. In the present study karyological screening of few animals revealed the presence of structural abnormalities like gaps and breaks. However, the frequency of such abnormalities was very low. None of the bull showed presence of any numerical chromosomal abnormalities.

CONCLUSION

Variation in the mean volume, mass activity, individual motility, sperm concentration and live sperm percent between bulls were non-significant. However, statistical variation for morphological abnormalities between breeding bulls was highly significant.

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REFERENCES

Ahmad, I., K. Javed and A. Sattar. 2004. Screening of breeding bulls of different breeds through karyotyping. *Pak. Vet. J.*, **24**(4): 190-192.

Bongso, T.A. and M. Hilmi. 1982. Chromosome banding homologies of a tendem fusion in river swamp and cross breed buffaloes (*Bubalus bubalis*), *Can. J. Genet. Cytol*., **24**: 667-673.

Campbell, R.C., H.M. Dott and T.D. Glover. 1956. The effect of exposure to high ambient temperature on spermatogenesis in the dairy bulls. *J. Dairy Sci*., **36**(4): 62-68.

Gunarajsingam, D., H. Abeygunawardena, U.V. Kuruwita, E.B.K. Perera and B.M.A.O. Perera. 1996. Seasonal variations in seminal and testicular characteristics in buffalo bulls.

Role of buffalo in rural development in Asia. p. 309-320. *In Proceedings of Regional Symposium,* Peradeniya, Sri Lanka.

Hafez, E.S.E. and B. Hafez. 2000. *Reproductive Cycle in Farm Animals*, **7**th ed. Lippincott Williams and Wilkins, Philadelphia, USA. p. 55-67.

Harman, H.A. and F.W. Madden. 1953. *The Artificial Insemination of Dairy Cattle-A Hand Book of Laboratory Manual*. Lucas Bros, Columbia, USA. 350p.

Kieffer, N.M. and T.C. Cartwright. 1968. Sex chromosome polymorphism in domestic cattle. *J. Hered.*, **59**(1): 34-36.

Mandal, A. and A. Sharma. 2003. Variations in the length of the Y chromosome and the seminal attributes of Karan fries bulls. *Vet. Res. Commun*., **27**(7): 567-575.

Moorhead, P.S., P.C. Nowell, W.J. Mellman, D.M. Battips and D.S. Hungerford. 1960. Chromosome preparations of leukocytes, cultured from human preparations blood. *Cell. Res*., **20**: 613-616.

Nath, R., S.S. Tripathi, V.B. Saxena and R.P. Tripathi. 1991. Tris diluents and freezability of buffalo semen. *Indian Vet. J*., **68**(2): 135-138.

Narayan, P., V.N. Reddy, P.A. Sharma, T.G. Honnappa, M. Devraj, A. Krishnaswamy and V.K. Arora. 1999. Spermiogram and biochemical studies in Murrah buffalo bulls. *Indian J. Anim. Reprod*., **20**(2): 156-158.

Pandey, A.K. 2001. *Effect of blood serum and caffeine on cryopreservation of buffalo spermatozoa*. M.V.Sc. Thesis, G.B. Pant University of Agriculture and Technology, Pantnagar, India.

Patel, K.V. 2011. Diagnostic and investigative andrology in crossbred bulls. *Indian J. Anim. Reprod*., **6**: 107-110.

Pattanayak, G., P.K. Rao, S.C. Mishra, K.C. Samantray and P. Manimaran. 2007. Screening of breeding bulls and cow through cytological investigation. p. 200-206. *In Compendium, 23rd Annual Convention of ISSAR and National Symposium*, QUAT, Bhubaneswar, India.

Perumal, P., A.K. Barik, D.N. Mohanty, R.K. Das and P.C. Mishra. 2009. Seminal characteristics of Jersey crossbred bulls. *In 25th Annual Convention of ISSAR and International Symposium*, Namakkal, India.

Shabd, R. 1998. *Cryopreservation of semen of Murrah, exotic and crossbred bulls*. M.V.Sc. Thesis, G.B. Pant University of Agriculture and Technology, Pantnagar, India.

Rao, A.R. 1971. *Changes in the morphology of sperm during the passage through the genital tract in bulls with normal and impaired spermatogenesis*. Ph.D. Thesis. Royal Veterinary College, Stockholm, Sweden.

Rodriguez, M.H. 2008. Estimation of fertility in breeding bulls. *In Proceedings of 15th International Congress on Biotechnology in Animal Reproduction*, Bangladesh Agricultural University, Mymen Singh, Bangladesh.

Selvaraju, S., I.J. Reddy, S. Nandi, S.B.N Rao and J.P. Ravindra. 2008. Influence of IGF-I on buffalo (*Bubalus bubalis*) spermatozoa motility, membrane integrity, lipid peroxidation and fructose uptake *in vitro*. *Anim. Reprod. Sci*., **113**(4): 60-70.

Shukla, M.K. 2002. *Studies on semen additives to improve cryopreservation of Murrah buffalo (Bubalis bubalis) semen*. M.V.Sc. Thesis. G.B. Pant University of Agriculture

and Technology, Pantnagar, India.

Shukla, M.K. and A.K. Misra. 2005. Correlations between seminal characteristics in Murrah bulls. *Indian J. Anim. Sci*., **75**(3): 263-266.

Siddique, R.A., G. Jagan, M. Mohanarao, R. Kumar, A. Kumar, P.K. Malik, C. Kumar and S.K. Atreja 2011. Sperm abnormalities and DNA fragementation *vis-à-vis* mammalian male infertility - A review. *Wayamba Journal of Animal Science*, **3**: 578-598.

Singh, P., S.K. Jindal, S. Singh and O.K. Hooda. 2000. Freezability of buffalo bull semen using different extenders. *Indian J. Anim. Reprod.*, **21**(1): 41-52.

Thiagarajan, V. 1987. *Karyological studies on buffaloes*. Ph.D. Thesis. Tamil Nadu Agricultural University, Coimbatore, Nadu, India.

Tiwari. M., R.B. Parsad and H.P. Gupta. 2009. Physico-morphology and *in vitro* fertility semen / spermatozoa of tarai buffalo semen. *Indian J. Anim. Physiol*., **1**: 11-14.

Veerabrahmaiah, K., V.H. Rao, A.S. Rao, K.V. Naidu and S.T.V. Rao. 2010. Semen characteristics of endangered Punganur bulls. p. 10-12. *In 26th Annual Convention of ISSAR and International Symposium*, G.B. Pant University of Agriculture and Technology, Pantnagar, India.

Willet, E.L. and P.J. Buckner. 1951. Determination of number of spermatozoa in bull semen by measurement of light transmission. *J. Anim. Sci*., **10**(1): 219-225.

Yadav, B.R. and C.R. Balakrishnan. 1982. On the y-chromosome of the Murrah buffaloes (*Bubalus bubalis*). *Can. J. Genet. Cytol.*, **24**: 189-192.

Yadav, B.R. 1981. *Studies on chromosomesand their abnormalities in cattle and buffaloes*. Ph.D. Thesis, Kurushsetra, Haryana, India.

Zemjenis, R. 1970. *Dignostic and Therapeutic Techniques in Animal Reproduction*, 2nd ed. Williams and Wilkins, Baltimore, Maryland, USA. 145p.