RESEARCH ARTICLE

Fresh and Post-Thaw Seminal Attributes of Jafarabadi Buffalo Bulls

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Abstract

The study was conducted on four Jafarabadi breeding bulls, 5-6 years old to know the fresh and post-thawed seminal characteristics based on total of 192 semen ejaculates evaluated and cryopreserved over one year period. The mean values of fresh neat seminal characteristics of Jafarabadi bulls, *viz*., ejaculate volume (ml), colour/density (score), sperm concentration (million/ml), mass activity (score), initial motility (%), live sperm (%), abnormal sperm (%), HOS reactive sperm (%) and acrosomal integrity (%) were 5.19±0.18, 2.38±0.10, 1253.36±24.75, 3.73±0.05, 80.31±0.05, 86.20±0.64, 5.00±0.40, 85.75±0.43 and 93.56±0.56, respectively, whereas the mean post-thawed sperm characteristics, *viz*., progressive sperm motility, live sperm (%), abnormal sperm (%), HOS reactive sperm (%), acrosomal integrity (%) and first insemination conception rate (%) observed were 57.60±0.36, 66.34±0.53, 8.85±0.33, 56.97±0.46, 75.26±0.17 and 44.63±0.14, respectively. The semen quality of fresh and post-thawed samples observed was within normal limit for use in breeding program with satisfactory first insemination conception rate.

Keywords: Acrosomal integrity, Conception rate, HOS reactive sperm, Jafarabadi bulls, Neat semen, Post-thaw seminal traits.

Ind J of Vet Sci and Biotech (2020): 10.21887/ijvsbt.15.3.12

Introduction

The production of spermatozoa is a continuous process in sexually mature bulls. The seminal traits of bulls reflect the status of testicular function and hormonal interrelationship. The seminal traits vary with the growth and maturity of animal, reproductive soundness, amount of testicular parenchymatous tissue and feeding regime. Correct determination of the number of sperm in neat as well as post-thaw semen is extremely important, as it is highly variable. When combined with volume of the ejaculate, this quantity of spermatozoa determines how many females can be inseminated from each ejaculate with the optimal number of sperm cells (Hafez and Hafez, 2000). Moreover, the information on neat as well as post-thaw seminal traits and conception rate of cryopreserved semen is meagre in Jafarabadi bulls (Rana and Dhami, 2004; Ghodasara *et al.,* 2016). So, the present investigation was carried out to determine different seminal attributes of fresh and post-thawed semen including conception rate with cryopreserved semen in Jafarabadi breed of buffalo.

Materials and Methods

The present study was carried out on semen ejaculates (192) collected by artificial vagina method in morning hours from four Jafarabadi breeding bulls and cryopreserved at Frozen Semen lab of Cattle Breeding Farm, JAU, Junagadh over one year period. The animals were fed as per the Minimum Standard Protocol (MSP-2000) decided by Government of India. The bulls were kept in sheltered paddocks with access to *ad libitum* water. The optimum health care was taken including clipping of preputial hair and vaccination against Haemorrhagic Septicaemia, Black Quarter and Foot and Mouth Disease. Besides this, annual screening of bulls for Tuberculosis, Johne's disease, Brucellosis, Campylobacteriosis and Trichomoniasis was also carried out. Immediately after collection, the ejaculates were assessed for volume (ml), colour (1=watery, 2=milky, 3=creamy), and sperm motility. A light microscope was used to determine mass activity (0-4 score) and the percentage of individual progressive motile spermatozoa. Sperm concentration as million per ml of semen was estimated using bovine photometer (Accucel, IMV, France) against 530 nM wave length. The morphological abnormalities of the spermatozoa were studied in the eosin-nigrosin stained slides. The acrosome integrity was evaluated by simplified nigrosine-eosin-Giemsa staining technique as described by Kutty *et al.* (1996), whereas the hypo-osmotic swelling test (HOST) was done using 150

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How to cite this article: Parmar, K.H., Kavani, F.S., Patbandha, T.K., Parikh, S.S., (2020). Fresh and Post-Thaw Seminal Attributes of Jafarabadi Buffalo Bulls. Ind J Vet Sci and Biotech, 15(3): 45-48.

Source of support: Nil

Conflict of interest: None.

Submitted: 09/01/2020 **Accepted:** 19/01/2020 **Published:** 10/03/2020

mosm/l solution to evaluate the functional integrity of the sperm membrane (Jayendran *et al.,* 1984). For freezing of semen Andromed extender and French medium straws were used employing conventional wide mouth liquid nitrogen freezer. The trial on first insemination conception rate was carried out on 800 Jafarabadi buffaloes inseminated with frozen-thawed semen of four bulls at Cattle Breeding Farm, JAU, Junagadh as well as in field during study period. The inseminated buffaloes were confirmed for pregnancy by per rectal palpation at 3 months post-insemination. The data was analyzed for calculating mean ± SE and significance by Statistical Packages for Social Sciences (IBM® SPSS® statistics, USA, version 20.0) software.

Results And Discussion

Ejaculate Volume

The mean semen ejaculate volume was found to be 5.13±0.10 ml with a range of 4.91 to 5.34 ml among four Jafarabadi bulls. This finding corroborated well with the reports on Indian buffalo bulls (Kerur *et al*., 1979) and Jafarabadi bulls (Rana and Dhami, 2004). However, lower semen volume in Murrah (Shukla and Mishra, 2005; Saini *et al*., 2017), Jafarabadi (Dhami and Shelke, 2005) and Nili-Ravi (Javed *et al*., 2000) bulls has been reported by others. The ejaculate volume is influenced by many factors, like the age, breed, body condition, scrotal size and weight, the reproductive health, method and frequency of collection, nutrition, season and management etc.

Semen Colour

The colour of semen in Jafarabadi bulls ranged from milky white to creamy white. The average score for semen colour was recorded to be 2.18±0.05 with a range of 2.08 to 2.29, indicating more inclination towards milky white colour. Similar milky white colour of semen in buffalo bulls of different breeds have been reported in many previous reports (Saini *et al*., 2017). The normal colour of semen found in all bulls during study suggested that the genital tracts of all the bulls were healthy without any infection or trauma and the ejaculates were free from any contaminants.

Sperm Concentration

The average sperm concentration was ascertained to be 1219.98±17.63 million/ml with a range varying from 1185.20 to 1254.76 million/ml among 4 bulls. This finding corroborated with the observations in Jafarabadi (Dhami *et al*., 2001) and Murrah bulls (Dhami and Sahni, 1994), whereas higher sperm concentration in Jafarabadi (Rana and Dhami, 2004) and Murrah (Selvaraju *et al.,* 2008), and lower mean sperm concentration in Murrah bulls (Bhatt *et al*., 2004) have also been reported. The concentration of spermatozoa varies with the sexual development and maturity of the bulls, with the feeding regime and with the reproductive health and size of the testis, season of the year and different geographical localities.

Mass Activity

The average mass activity score of Jafarabadi bull semen was recorded to be 3.72±0.03 with a range of 3.66 to 3.78 among 4 bulls, indicating very good motility with waves and eddies formation. Similar mass activity was reported in Murrah bulls by Shukla and Mishra (2005). However lower mass activity in Jafarabadi (Rana and Dhami, 2004) and Murrah bulls (Bhatt *et al*., 2004) have also been reported. The mass motility is affected by many factors like season, age of the bull, the frequency of collection, the degree of stimulus provided and type of thrust. The variation in result may also be attributed to difference in judgment of mass activity, total number of observations made and climatic condition.

Initial Motility

The per cent initial sperm motility was in the range of 78.85 to 80.20 with an average of 79.53±0.34 among 4 bulls (Table 1). This was in agreement with the earlier report in Jafarabadi bulls (Dhami and Shelke, 2005). However, higher initial motility in Murrah (Saini *et al.,* 2017) and Jafarabadi bulls (Dhami *et al.,* 2001) have been reported by others, while Selvaraju *et al.* (2008) recorded relatively lower initial motility in Murrah bulls. The initial sperm motility is an important attribute for acceptance or rejection of the ejaculate for further processing and use in AI. The variation observed in the initial sperm motility is attributed to degree of sexual excitement, method of semen collection, frequency of semen collection and such other factors.

Live Sperm Count

The mean per cent live sperm in neat semen of four Jafarabadi bulls was in the range of 83.26 to 85.25 with an average of 84.26±0.50. It corroborated with earlier observations in Jafarabadi bulls (Dhami and Shelke, 2005), while lower live sperm per cent in Murrah bulls was found by Saini *et al*. (2017). The optimum live sperm count might be due to use of bulls of known good fertility and maintenance of identical and

Table 1: Sperm quality parameters (Mean±SE) of fresh and frozen-thawed semen of Jafarabadi bulls

S. No.	*Sperm quality attributes*	*Fresh semen*	*Post-thaw semen*
1	Progressive sperm motility (%)	79.53 ± 0.34	57.60 ± 0.36
2	Live spermatozoa (%)	84.26 ± 0.50	66.34 ± 0.53
3	Abnormal spermatozoa (%)	6.06 ± 0.33	8.85 ± 0.33
4	HOS reactive sperm (%)	85.46 ± 0.31	56.97 ± 0.46
5	Acrosome integrity (%)	92.06 ± 0.20	75.26 ± 0.17

optimal conditions of feeding and management throughout the study period.

Abnormal Sperm Count

The per cent abnormal sperm varied from 5.39 to 6.72 with an overall mean of 6.06±0.33 in the fresh semen of four Jafarabadi bulls (Table 1). Similar findings in Nili-Ravi bulls (Javed *et al*., 2000) have been reported. However, Dhami *et al*. (2001) and Saini *et al*. (2017) reported higher mean abnormal sperm per cent in Jafarabadi and Murrah bulls, respectively. The sperm abnormalities may be either hereditary or arise because of defects caused by infectious diseases or environmental factors.

HOS Reactive Sperm

The per cent HOS reactive spermatozoa varied from 84.85 to 86.08 with an overall mean of 85.46±0.31 in the fresh semen of Jafarabadi bulls. However, Rana and Dhami (2004) reported lower per cent of HOS reactive spermatozoa in same breed.

Acrosome Integrity

The values of acrosome integrity varied from 91.65 to 92.47 with an overall mean of 92.06±0.20 per cent in the fresh semen of Jafarabadi bulls (Table 1). However, it was higher than the values reported for same breed by Rana and Dhami (2004) and Dhami and Shelke (2005).

Post-Thaw Sperm Motility

The per cent post-thaw sperm motility varied from 56.88 to 58.32 with an overall mean of 57.60±0.36 in the Jafarabadi bulls studied (Table 1). It was similar to the report in Murrah bulls (Tiwari *et al*. 2011). However, slightly higher post-thaw motility in Murrah and Surti bulls (Pathak *et al*., 2018) and Jafarabadi bulls (Ghodasara *et al*., 2016) has been reported by others. The post-thaw motility is the most common parameter to assess the effects of freezing on spermatozoa and also as an indicator governing the use or discard of semen for artificial insemination. Post-thaw motility rates vary from 30-70 per cent and are known to be influenced by a number of factors such as extenders, cryoprotectants, freezing procedures, age of the bull and season of semen collection.

Post-Thaw Live Sperm

The overall mean per cent post-thaw live sperm was 66.34±0.53 and it ranged from 65.27 to 67.40 in Jafarabadi bulls, which was in line with earlier report on same breed by Rana and Dhami (2004), while slightly higher value was reported in Surti and Murrah bulls by Pathak *et al*. (2018). The variations might be due to different geographical location, different breed, experimental design, and protective measures provided during experimental period.

Post-Thaw Abnormal Sperm

The per cent post thaw abnormal sperm varied from 8.19 to 9.51 with an overall mean of 8.85 ± 0.33 in semen of Jafarabadi bulls (Table 1). Similar post-thaw abnormal sperm count was reported by Bhakat *et al.* (2015) in Murrahs, whereas higher count was found in Jafarabadi bulls by Rana and Dhami (2004). During semen processing (dilution, equilibration and freezing) the abnormal sperm count increases significantly. However, post-thaw abnormal sperm count remained within the permissible limit in the present study.

Post-Thaw HOS Reactive Sperm

The per cent HOS reactive spermatozoa varied from 56.06 to 57.89 with an overall mean of 56.97±0.46 in the post-thaw semen of Jafarabadi bulls. However, Pathak *et al*. (2018) recorded lower per cent mean post-thaw HOS reactive spermatozoa in Surti and Murrah bulls. This variation could be due to the difference in the freezing methods, extender, thawing rate, and method of measurement.

Post-Thaw Acrosome Integrity

The per cent acrosome integrity of spermatozoa varied from 74.91 to 75.60 with an overall mean of 75.26±0.17 in the post-thaw semen of Jafarabadi bulls (Table 1). The present finding was according to observation on Murrah buffalo bulls (Rasul *et al*., 2000), whereas lower post-thaw acrosome integrity of sperm have been reported by Bhakat *et al.* (2015). The variation might be due to the damage during dilution, cooling, freezing and thawing process.

First AI Conception Rate

The mean first artificial insemination (AI) conception rate of Jafarabadi bull semen was found to be 44.63±0.14 % with a range of 44.34 to 45.91%. This was comparable with findings in Murrah and local buffaloes (Guangsheng *et al*., 2013; Ghuman and Dhami, 2017;), while lower conception rate in Nili-Ravi (Younis *et al*., 1999) and Murrah buffaloes (Gokhale and Bhagat, 2000), and higher conception rate in Nili-Ravi buffalo (Guangsheng *et al*., 2013) have been reported by some workers.

The conception rates in artificially inseminated bovines varied considerably across species, breeds and geographical locations. These could have been due to multiple animals, environmental factors, expression and detection of heat symptoms, AI technicians, semen handling from collection, processing to cryopreservation and thawing, individual female fertility, embryonic mortality and such other factors.

Acknowledgement

The authors are thankful to the authorities of Junagadh Agricultural University, Junagadh for granting permission to perform the research and providing necessary facilities.

References

Bhakat, M., Mohanty, T.K., Gupta, A.K., Prasad, S., Chakravarty, A.K. and Khan, H.M. (2015). Effect of season on semen quality parameters in Murrah buffalo Bulls. *Buffalo Bulletin,* **34**(1): 100-112.

Bhatt, V., Honnappa, T.G. and Dubey, B.M. (2004). Seasonal effects on seminal attributes in Murrah bulls under Bangalore agro-climatic conditions. *Indian J. Anim. Reprod.*, **25**(1): 23-24.

Dhami, A.J. and Sahni, K.L. (1994). Comparative appraisal of physico-morphological and enzymatic attributes of semen and their interrelationships in ox and buffalo. *J. Appl. Anim. Res.,* **5**(1): 13-20.

Dhami, A.J. and Shelke, V.B. (2005). Investigations into the causes of poor semen quality and freezability in Jafarabadi buffalo bulls. *The Indian J. Anim. Sci.,* **75**(8): 925-929.

Dhami, A.J., Shelke, V.B., Dave, R.R. and Kavani, F.S. (2001). Scrotal biometry, sexual behaviour and semen production in breeding bulls of different breeds – A comparative study. *Indian J. Dairy Sci.,* **54**: 255-260.

Ghodasara, S.N., Gajbhiye, P.U., Ahlawat, A.R. and Murthy, K.S. (2016). Evaluation of fresh semen quality and predicting the number of frozen semen doses in Jafarabadi buffalo bull. *Buffalo Bulletin,* **35**(1): 65-72.

Ghuman, S.P.S. and Dhami, D.S. (2017). Seasonal variation in AI and pregnancy rate in buffalo and improving their fertility status following application of FTAI during non-breeding season. *Indian J. Anim. Reprod.,* **38**(1): 4-8.

Gokhale, S.B and Bhagat, R.L. (2000). Status of reproductive performance in rural buffaloes artificially inseminated using deep frozen semen. *Indian J. Anim. Sci.,* **70**: 366-368.

Guangsheng, Q., Bingzhuang, Y., Zhengzhun, T., Hui, L.I., Jian, H. and Xianwei, L. (2013). Effect of frozen semen from Italian Mediterranean buffalo on some reproductive parameters and conception rate performed to different species of water buffalo in Southern China Guangsheng. *Buffalo Bulletin,* **32**(2): 577-581.

Hafez, E.S.E. and Hafez, B. (2000). *Reproductive Cycle in Farm Animals*, 7th Edn., Publ., Lippincott Williams and Wilkins, Philadelphia, USA, pp 55-67.

Javed, M.T., Khan, A. and Kausar, R. (2000). Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. *Veterinarski Arhiv,* **70**(2): 83-94.

Jeyendran, R.S., Vandervent, H.H., Perz-Pelaez, Z.M., Crabo, B.G. and Zanevald, L.J.D. (1984). Development of an assay to assess the functional integrity of human sperm membrane and its relationship to their semen characteristics. *J. Reprod. Fert.,* **70**(1): 219-228.

Kerur, V.K., Serasia, A.H. and Bhavsar, B.K. (1979). Effect of vitamin-A feed supplementation on semen production of Jafarabadi buffalo bulls. *Indian Vet. J.*, **56**: 1020-1022.

Kutty, I.C., Joseph, M. and Iyer, C.P.N. (1996). A simplified nigrosine-eosin-giemsa staining technique to distinguish acrosome damaged live and dead spermatozoa. *Indian J. Anim. Res.*, **17**(2): 122-123.

Pathak, A.K., Dhami, A.J. and Chaudhary, D.V. (2018). Seminal attributes, freezability and their interrelationships in Zebu cattle and buffalo bull from central Gujarat. *The Indian J. Vet. Sci. & Biotech.*, **14**(2): 59-62.

Rana, C.M. and Dhami, A.J. (2004). Physical attributes intact acrosome, HOS test and freezability of semen of Gir and Jaffarabadi bulls. *Indian Vet. J.*, **81**: 406-410.

Rasul, Z., Anzar M., Jalali, S. and Ahmad, N. (2000). Effect of buffering system on post-thaw motion characteristics, plasma membrane integrity and acrosome morphology of buffalo spermatozoa. *Anim. Reprod. Sci.,* **59**(1-2): 31-41.

Saini, J., Dhande, P.L., Gaikwad, S.A., Shankhapal, V.D. and Shelar, R.R. (2017). Physico-morphological characters of semen of Murrah buffalo bull. *Indian J. Anim. Reprod.,* **38**(1): 54-55.

Selvaraju, S., Ghose, J., David, C.G., Reddy, I.J. and Ravindra, J.P. (2008). Sperm nuclear morphology in relation to sperm functional tests in assessing buffalo semen. *Indian Vet. J.*, **85**: 505-507.

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

Tiwari, R., Mishra, G.K., Shukla, M.K., Singh, R.B., Saxena, S.K. and Siddiqui, M.U. (2011)**.** Seasonal variations in semen production of Murrah buffalo bulls. *Indian J. Anim. Reprod.,* **32**(2): 5-7.

Younis, M., Samad, H.A and Ahmad, N. (1999). Fertility of frozen-thawed semen collected from young, adult and old buffalo bull during low and peak breeding seasons. *Pakistani Vet. J.*, **19**: 78-80.