BREED INFLUENCE ON QUALITY OF FROZEN BULL SEMEN ASSESSED BY *IN VITRO* FERTILITY TESTS

Alanteena Jacob[1], Metilda Joseph[2], K. N. Aravinda Ghosh[3], M.O. Kurien[4], Hiron M. Harshan[5], V. Ramnath[6] and Jose James[7]
Department of Animal Reproduction, Gynaecology and Obstetrics,
College of Veterinary and Animal Sciences,
Mannuthy,Thrissur, Kerala.

Received -05.07.2016
Accepted -05.07.2016

Abstract

Four bulls each of four breeds (crossbred Holstein Friesian, crossbred Jersey, Vechur and Kasargod Dwarf) used for frozen semen production in Kerala Livestock Development Board were selected to evaluate the effect of breed on the quality of frozen semen. Semen samples of all the bulls (n=16) procured monthly for a period of ten months were subjected to in vitro quality assessment tests. Among the seven quality parameters studied, only progressive motility and cervical mucus penetration distance of post thaw spermatozoa were observed to be significantly ($p<0.05$) influenced by breed of the bull. There was no significant influence of breed on viability, abnormality, acrosome integrity, functional membrane integrity and induced acrosome reaction of frozen thawed spermatozoa. The results revealed the influence of breed of bull on selected quality parameters of frozen semen.

Key words: *Frozen bull semen, breed, in vitro fertility, cervical mucus penetration distance, in vitro capacitation*

Introduction

The success of artificial insemination programme in cattle is mainly dependent on quality production of frozen semen, as it is used extensively for cattle breeding programmes. Even though quality control measures for frozen semen production are strictly followed as per the Minimum Standards for Protocols, the outcome is not reaching the expected levels.

The cattle breeding policy of Kerala is crossbreeding, hence majority of the cattle

1. MVSc Scholar and corresponding author
2. Associate Professor,
3. Former Professor and Head,
4. Professor and Head,
5. Assistant Professor
6. Controller of Examinations & Implementing Officer, Central Instruments Laboratory, KVASU
7. Managing Director, Kerala Livestock Development Board Ltd.
Email: alanteenajacob@gmail.com :Phone no: 9497654997

population are crossbreds. Even though the number of indigenous animals is less, there are two unique cattle breeds- Vechur and Kasargod Dwarf- which are highly adapted for the specific climatic conditions of Kerala. Recent studies using fresh semen showed a significant influence of cattle breed on *in vitro* fertility of spermatozoa (Boujenane and Boussaq, 2014). But research works to assess the influence of this factor on frozen semen are few. Hence, the present study was carried out to evaluate the genetic influences on frozen semen quality by applying various *in vitro* fertility tests, with the ultimate objective of strengthening of artificial insemination programme in Kerala.

Materials and Methods

Procurement of frozen semen samples

This study was conducted to evaluate the effect of breed on the quality of frozen bovine semen using *in vitro* fertility tests. Four animals each of four breeds, Crossbred Holstein Friesian (CBHF- Holstein Friesian X Gir), crossbred Jersey (CBJY- Jersey X Red Sindhi), Vechur (VR) and Kasargod Dwarf (KD), with age at collection averaged approximately 50 months were selected for the present study. The selected animals were maintained at bull station of KLDB, Dhoni, Palakkad and used for regular semen collection. Frozen semen samples of each bull produced during each month were procured at regular intervals for a period of 10 months and subjected to various *in vitro* fertility tests. French mini straws (0.25mL) containing a dose of 20 million spermatozoa diluted and frozen stored in TRIS-citrate-fructose-egg yolk-glycerol extender, were used for the experiment.

Post thaw semen evaluation

Routine sperm parameters: Thawing of semen was carried out in a water bath at 37°C for 30 seconds. Per cent progressive motile spermatozoa were assessed using phase contrast microscopy (400X). Eosin-Nigrosin staining technique was employed for evaluating the viability and abnormality of spermatozoa in the sample.

Sperm function tests: Intact acrosome per cent was assessed as described by Watson (1975). Hypo-osmotic swelling test (HOST) for assessing the per cent of spermatozoa with plasma membrane integrity was conducted with the test solution of 150 mOsm/L (Jayendran *et al.,*1984) and the per cent of spermatozoa responded to hypo-osmotic medium were recorded.

Fertility tests: Bovine cervical mucus penetration test was used to assess the ability of frozen thawed spermatozoa to penetrate the mucus barrier in the female genital tract. To reduce the variation in the results bovine cervical mucus was substituted with two per cent polyacrylamide gel prepared using 1.5 *M* TRIS buffer (Anilkumar *et al.,* 2001). The test was conducted by keeping two of the non-heparinized hematocrit capillary tubes (75 mm length and 0.8 mm diameter) in semen reservoir for 30 minutes at 37°C in an incubator. The distance travelled by the vanguard spermatozoa was measured using 400X objective of phase contrast microscope. The result was expressed in mm/30 min.

The *in vitro* capacitation status was assessed indirectly by inducing acrosome reaction in the capacitated spermatozoa (Parrish *et al*., 1988). Thawed semen samples from 0.25 mL French mini straws containing 20 million spermatozoa were transferred to the Eppendorf's tubes. In order to remove the extender, the samples were washed by diluting 0.25 mL semen with one mL Sp-TALP (Tyrode's albumin lactate pyruvate), centrifuged at 275 g for 10 min at 20-25^{0}C, resuspended the sperm pellet in 1.25 mL Sp-TALP, and repeated the centrifugation. The resulting sperm pellet was resuspended in one mL Sp-TALP, heparin was added at 10 µg/mL and the samples were kept in a CO_2 incubator for 4 h with five per cent CO_2 in air and at 39^{0}C. At the end of incubation, lysophosphatidylcholine was added at 100 µg/mL and the sperms were incubated for an additional 15 minutes. Trypan blue- Giemsa double staining (Kovacs and Foote, 1992) was used to evaluate the percent of live acrosome reacted spermatozoa which was assessed under 400X magnification of a bright-field microscope.

Statistical analysis

Effect of breed of the bull on *in vitro* fertility of spermatozoa was evaluated by subjecting the collected data to statistical analysis using SPSS version 21 software. Repeated measures ANOVA was used to compare the means.

Results and Discussion

Routine sperm parameters:Post-thaw motility of frozen thawed spermatozoa was found to be significantly influenced by breed of the bull (Table 1). Similar to the finding of the present study, Boujenane and Boussaq (2014) reported that breed of bulls had significant effect on post thaw motility. Among the four breeds CBJY semen showed significantly lowest ($P<0.05$) overall mean post thaw motility than the semen of other three breeds which were having statistically similar mean post thaw motility. Similarly, lower values were also reported by Tuli *et al.* (1988) and Kumar *et al.* (2015) in CBJY bulls.

Non-significantly higher overall mean per cent for post thaw viability (63.42±2.47) were observed in CBHF semen samples, whereas those values were non-significantly lower for the indigenous bull semen.

The post thaw motility and viability per cent obtained for frozen semen of indigenous breeds in Kerala were similar to that reported in other indigenous breeds like Gir (Chowdhury *et al.*, 2013) and Kankrej (Patel and Siddiquee, 2013). Post thaw viability per cent observed in semen of cross bred Jersey bull (Kumar *et al.*, 2015) and pure bred bulls (Zodinsanga *et al*., 2015) in earlier studies were 51.63±0.97 and 59.9±1.9 respectively which were comparable to the results obtained in the present study.

Non-significantly lowest mean per cent post thaw total sperm abnormalities were observed in KD semen. Whereas non-significantly high spermatozoa abnormality was noticed in CBJY and VR bull semen. The sperm abnormality per cent observed in the frozen semen samples were lower than the recommended permissible limit. It indicated the good quality of these samples.

Sperm function tests: Non-significantly higher mean per cent post thaw acrosome intactness was observed in KD semen, whereas non-significantly low acrosome integrity was noticed in CBJY and VR bull semen. The per cent of acrosome intactness of frozen thawed spermatozoa of different breeds under study were found to be higher than that obtained for Kankrej bulls (Patel and Siddiquee, 2013) and crossbred Jersey bulls (Kumar *et al.,* 2015). But those were lower than the values reported by Zodinsanga *et al*. (2015) in pure bred (79.9±3.8) and cross bred (92.9±2.4) bulls.

Functional plasma membrane integrity assessed through HOST was non-significantly higher in frozen semen of CBJY bulls and lower in VR bulls. All the values of HOST response noticed in this study were higher than the

Table 1. Effect of breed on post thaw parameters of frozen bovine semen (Mean±SE)

Post thaw semen parameters	CBHF (n=40)	CBJY (n=40)	VR (n=40)	KD (n=40)
Motility (%)	55.47 ± 2.82^{ab}	44.01 ± 5.80^{c}	53.44 ± 3.56^{ab}	56.41 ± 6.12^{a}
Viability (%)	63.42 ± 2.47^{ns}	59.62 ± 3.98^{ns}	57.24 ± 3.11^{ns}	57.61 ± 2.33^{ns}
Abnormality (%)	2.91 ± 0.71^{ns}	3.65 ± 1.47^{ns}	3.36 ± 1.20^{ns}	2.36 ± 1.49^{ns}
Acrosome integrity (%)	78.23 ± 1.43^{ns}	71.76 ± 2.59^{ns}	71.86 ± 1.71^{ns}	80.14 ± 3.73^{ns}
Response to HOST (%)	63.57 ± 1.61^{ns}	68.87 ± 1.00^{ns}	61.23 ± 3.02^{ns}	63.91 ± 4.98^{ns}
Gel penetration distance (mm/30 min)	12.34 ± 0.30^{ab}	12.28 ± 0.50^{abc}	11.53 ± 0.21^{bc}	13.09 ± 0.49^{a}
Acrosome reacted sperms (%)	48.70 ± 6.46^{ns}	46.60 ± 4.69^{ns}	43.32 ± 4.82^{ns}	42.42 ± 3.82^{ns}

Values with different superscripts within the same row (a,b,c,d) differ significantly at 5% level

minimum recommended percent (minimum 40) and the previously reported plasma membrane integrity of various breeds of bulls (Chowdhury *et al.*, 2013; Kumar *et al.*, 2015 and Zodinsanga *et al.*, 2015).

Fertility tests: The distance covered by frozen thawed spermatozoa in polyacrylamide gel was significantly lower ($P<0.05$) for VR semen than KD semen. The crossbreds showed statistically similar values with that of KD in this test. The values obtained in this study were in agreement with the observations of Becher *et al*. (2013) but lower than that reported by Anilkumar *et al.* (2001) in bull semen using similar technique. Perusal of literature did not reveal any effect of breed of the bull on gel penetration ability of the frozen thawed spermatozoa.

Non-significantly higher overall mean per cent for induced acrosome reaction (48.70±6.46) were observed in CBHF semen samples, whereas those values were non-significantly lower for the indigenous bull semen.

In contrast to the present study, Demyda-Peyras *et al.* (2012) reported that there was significant influence of breed of various endangered Spanish bulls on induced *in vitro* acrosome reaction in frozen semen samples. The per cent of post thaw bovine spermatozoa that were acrosome reacted *in vitro*, was lower than that observed by Parrish *et al.* (1988) and Costa *et al.* (2010) and higher than the values reported by Zodinsanga *et al*. (2015) who induced *in vitro* capacitation and acrosome reaction in spermatozoa with the same tech nique.

The results of the present study were confirming the effect of breed of bull, on certain quality parameters of frozen semen. Post-thaw motility and gel penetration distance of frozen thawed spermatozoa were found to be significantly influenced by breed of the bull (Table 1). But values obtained for all the other post thaw semen parameters did not differ significantly between the four breeds under study. Among crossbreds, CBHF was found to be the better frozen semen producer whereas among indigenous breeds Kasargod Dwarf was observed to be superior. Vechur and Kasargod Dwarf breeds are under conservation and selection intensity of these bulls for freezing of semen are low when compared to cross breds. Employing superior bulls of selected breeds by careful semen quality analysis in the crossbreeding programme, along with the conservation and intense selection of indigenous bulls will help to satisfy the needs of bovine assisted reproductive programmes in the state.

References

Anilkumar, R., Devanathan, T.G., Pattabiraman, S.R. and Edwin, M.J., 2001. Correlation between the spermatozoal characteristics and sperm penetration distance in polyacrylamide gel and bovine cervical mucus. *Theriogenology.* **55**: 685–691.

Becher, A. C., Failing. K., Kauffold, J. and Wehrend, A. 2013. Establishment of a practical sperm penetration test for bovine semen. *Tierärztl Prax*. **41**: 297–303.

Boujenane, I. and Boussaq, K. 2014. Breed effects on semen traits of dairy and beef artificial insemination bulls. *Livest. Res. Rural Dev.* **26**: 114-118.

Chowdhury, S., Das, S., Gupta, T., Sana, D. and Bose, S. 2013. A comparative study with Murrah buffalo and indigenous Gir spermatozoa to Hypo-Osmotic Swelling Test (HOST). *Explor. Anim. Med. Res*. **3**: 159-164.

Costa, M.Z., Oliveira, L.Z., Resende, M.V., Lucio, A.C., Perini, A.P., Miguel, M.C.V. and Lima, V.F.M.H. 2010. Induction of the acrosome reaction test to *in vitro* estimate embryo production in Nelore cattle. *Arq. Bras. Med. Vet. Zootec.* **62**: 771-777.

Demyda-Peyras, S., Dorado, J., Hidalgo, M., DeLuca, L., Mun˜oz-Serrano, A. and Moreno-Millan, M. 2012. *In vitro* induction of the acrosome reaction in spermatozoa from endangered Spanish bulls: Effect of breed, culture media and

incubation time. *Livest. Sci*. **149**: 275–281.

Jeyendran, R.S., Van der Ven, H.H., Perez-Pelaez, M., Crabo, B.G. and Zaneveld, L.J.D. 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J. Reprod.Fert*. **70**: 219-228.

Kovács, A. and Foote, R. H. 1992. Viability and acrosome staining of bull, boar and rabbit spermatozoa. *Biotechnic Histochemistry.* **67**: 119–124.

Kumar, U., Gawande, A.P., Sahatpure, S.K., Patil, M.S., Lakde, C.K., Bonde, S.W., Borkar, P.L., Poharkar, A.J. and Ramteke, B.R. 2015. Assessment of semen quality in pure and crossbred Jersey bulls. *Vet.Wld*. **8**: 1266-1272.

Parrish, J.J., Susko-Parrish, J.L., Winer, M.A. and First, N.L. 1988. Capacitation of bovine sperm by heparin. *Biol. Reprod*. **38**: 1171-1180.

Patel, B.R. and Siddiquee, G. M. 2013. Physical and morphological characteristics of Kankrej bull semen. *Vet Wld*. **6**: 405-408.

Tuli, R.K., Batabyal, A.K., Singal, S.P. and Razdan, M.N. 1988. Seminal characteristics and deep freezing of CB bull semen using Tris yolk glycerol extender. *Indian J.Anim.Reprod.* **9**: 16-18.

Watson, P.F. 1975. Use of Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. *Vet Rec*. **97**: 12–15.

Zodinsanga, V., Mavi, P.S., Cheema, R.S., Kumar, A. And Gandotra V.K. 2015. Relationship between routine analysis, sperm function and fertility tests of cattle bull semen. *Asian J. Anim. Sci.* **9**: 37-44. ■