Original Research

Cryopreservation Induced Alteration in Sperm Morphology in Hariana Bull Semen

Chetna Gangwar[1]*, Atul Saxena[2], P. K. Shukla[3], S. P. Singh[4], Akhil Patel[5], Mohit Antil[6] and Abhinov Verma[7]

[1]ICAR- Central Institute for Research on Goats, Makhdoom, Mathura, Uttar Pradesh- 281 122, INDIA
[2]Department of ARGO, College of Veterinary Sciences, DUVASU, Mathura, Uttar Pradesh-281001, INDIA
[3]DUVASU, Mathura, Uttar Pradesh- 281001, INDIA
[4&7]Department of Anatomy, College of Veterinary Sciences, DUVASU, Mathura, Uttar Pradesh-281001, INDIA
[5]Department of ARGO, College of Veterinary Sciences, SVPUA&T Meerut, Uttar Pradesh, INDIA
[6]ICAR- IVRI (CIRG) Mathura, Uttar Pradesh- 281 122, INDIA

***Corresponding author:** chetnaom82@gmail.com

Rec. Date:	Aug 27, 2018 06:06
Accept Date:	Oct 12, 2018 13:05
DOI	10.5455/ijlr.20180827060644

Abstract

Present study was conducted on 04 Haryana bulls to evaluate the effect of semen cryopreservation on sperm morphology. Thirty two semen ejaculates, 08 from each bull were collected using Artificial Vagina method at weekly interval. After preliminary semen evaluation, suitable semen samples were diluted with glycerolated egg yolk TRIS diluter. Semen samples were cryopreserved following the equilibration for 4hrs. at 5˚C and finally stored into liquid nitrogen container. For sperm morphology head length, head width, base width, head area, mid piece length, tail length, total length, head ellipticity, head elongation and head regularity was measured in fresh as well as cryopreserved semen samples. Findings of this study revealed that there is a significant (P< 0.05) reduction in head area and other measurements of spermatozoa after cryopreservation. It may be concluded that cryopreservation induces various morphological changes in sperm which might be responsible for reduced fertility of frozen thawed spermatozoa.

Key words: Cryopreservation, Fertility, Hariana Bull, Sperm Morphometry

How to cite: Gangwar, C., Saxena, A., Shukla, P., Singh, S., Patel, A., Antil, M., & Verma, A. (2019). Cryopeservation Induced Alteration in Sperm Morphology in Hariana Bull Semen. International Journal of Livestock Research, 9(2), 282-288. doi: 10.5455/ijlr.20180827060644

Introduction

Assessment of semen quality is frequently done by evaluation of sperm concentration, motility, viability and acrosomal integrity but sometimes sires with evidently normal semen that is showing lower fertility results, provide the propulsion for further studies to illuminate other markers of fertility. Sperm head

morphology has been reported as pivotal indicator of fertility (Kipper *et al.*, 2017). Head shape and sperm morphometry along with other basic seminal traits can be used to make an overall fertility index and could have the potential to rank sires according to their fertilizing ability. Biophysical coalitions between sperm morphology and motility may influence the movement of spermatozoa through the female reproductive tract, but unpredictability remain regarding how sperm morphology influences the fertilization event, and whether only the sperm measurements in itself are involved (Francisco *et al.*, 2016). The morphometric interferences appearing during cryopreservation process may be related to osmotic stress, cold shock, intracellular ice crystal formation, chemical stress, excessive production of reactive oxygen species (ROS) and alteration in antioxidant defense systems or combinations of these conditions. It can be minimized by use of antioxidants such as Vitamin C and glutathione in semen (Gangwar *et al.*, 2015; Gangwar *et al.*, 2018). In some species morphometry is particularly indispensable since sperm head shape and size relates to fertility (Ostermeier *et al.*, 2001) or freezability (Thurston *et al.*, 2001; Pena *et al.*, 2005).

Further exploration regarding channel involved thereof and the prevention of early membrane modifications will optimistically lead to the refinement of the fertility of cryopreserved semen (Andrabi, 2009). Therefore, there is a need to discuss in acuity the major components influencing the successful cryopreservation of cattle bull semen. The aim of this study was to evaluate the effect of freeze-thawing procedure on sperm head morphometry in Hariana bulls and whether it can be used as a marker, together with traditional semen evaluation methods of the sperm freezability and the potential fertility of the cryopreserved sperm.

Materials and Methods

Experimental Animals and Design

Present study was conducted on four Hariana bulls having age group between 6.5–7.5 years and weighing about 450-500 kg body weight, reared at the Semen Biology Lab, University Instructional Livestock Farm Complex (ILFC), College of Veterinary Sciences, U. P. Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh which is located in a semiarid zone of Northern part of India and experiment was conducted as per the guidelines of institutional ethical committee.

Semen Collection

Semen was collected biweekly from individual bull with the help of separate artificial vagina (AV) between 7.0 to 8.0 AM. Immediately after collection, tube containing semen were marked and placed in the water bath at 35°C. A total of 32 ejaculates from 4 bulls or 8 ejaculates from each bull were collected. After initial semen evaluation sperm morphometry was done in fresh as well cryopreserved semen. Total 128 live sperm

 DOI 10.5455/ijlr.20180827060644

per bull were used for sperm biometry estimation from various fields on four slides prepared for each bull and each ejaculate.

Evaluation of Semen

Immediately after semen collection, the volume and pH of each ejaculate was recorded. For evaluation of mass motility (0–5 scale), a drop of freshly collected semen was put over a clean grease free slide maintained at 37°C and examined under low power (10×) of the microscope. For evaluation of progressive motility, a drop (10 µl) of diluted semen was placed on a clean grease free slide maintained at 37°C covered with a cover slip and examined under phase contrast microscope at 40× magnification. The ejaculates with a concentration of more than 600 million spermatozoa per ml and more than 70 % progressive forward motility were used for the experiment (Gangwar *et al.*, 2014).

Sperm Morphometry

A drop semen was mixed with two or three drops of eosin-nigrosin stain (100 mg eosin + 500 mg nigrosin dissolved in 10 mL of 2.9% sodium citrate buffer, pH 6.9) and placed on a clean glass slide at 37 °C. Sperm were examined (1000 X magnification) in fresh as well as cryopreserved samples with the help of computerized Phase Contrast Leica DM750 microscope. The system was first calibrated with images of standard length for known magnifications and measurement accuracy of 0.1 µm. Each sperm head was measured for four primary parameters [head area ($A=1.05-0.081xB^2+0.64xWxL$) μm^2, head length (L) µm, head width (W) µm, head base (B) width µm] and three derived parameters of head shape [ellipticity (L/W), elongation ((L - W)/(L + W)), regularity (π LW/4A)]. Other spermatozoa dimensional characteristics (midpiece length, tail length and total sperm length) were also studied. Head shape was determined by dividing the head length by head breadth (Gravance *et al.*, 1998).

Semen Cryopreservation and Thawing

After morpho-physiological evaluation and those confirming the criteria for freezing, fresh ejaculate was extended (at 35° C) to fix the concentration at 80 million spermatozoa/ml. The filled and sealed straws (French mini straws, IMV, France) were kept in cold handling cabinet for 4 h at 4°C. Straws were vapour frozen in automatic biological freezer and then the cryopreserved straws were stored in liquid nitrogen. The frozen semen was thawed in thawing unit (IMV, France) maintained at 37° C for 45 seconds.

Statistical Analysis

For the present study 128 sperm from each Hariana bull considered for morphometry. The raw data were analyzed using statistical package SPSS V 20 and expressed in the form of mean and standard error of

 DOI 10.5455/ijlr.20180827060644

mean. Comparisons were made by employing paired t- test. Homogeneity test was also conducted to assign different superscript to variables based on significant difference at $P < 0.05$ between different variables.

Results and Discussion

In the present study head length, width, base width, head area, midpiece length, tail length, total length, head regularity, head ellipticity and head elongation of spermatozoa significantly ($P < 0.05$) decreased after cryopreservation.

Table 1: Comparison of sperm morphometry (µm) in fresh and post thaw semen of Hariana bull

Parameters	Fresh	Post-thaw	't' value
Head length	$10.19^{b} \pm 0.04$	$9.32^{a} \pm 0.03$	**16.75****
Head Width	$4.57^{b} \pm 0.02$	$4.40^{a} \pm 0.02$	**5.24****
Base diameter	$2.51^{b} \pm 0.08$	$2.44^{a} \pm 0.02$	**2.88****
Head area (μm^2)	$30.32^{b} \pm 0.18$	$26.84^{a} \pm 0.17$	**14.15****
Mid piece length	$13.24^{b} \pm 0.08$	$12.87^{a} \pm 0.05$	**3.69****
Tail length	$46.44^{b} \pm 0.20$	$45.81^{a} \pm 0.14$	**2.66****
Total length	$69.70^{b} \pm 0.23$	$68.01^{a} \pm 0.14$	**6.58****
Head regularity	$1.20^{b} \pm 0.0003$	$1.20^{a} \pm 0.0004$	**7.90****
Head ellipticity	$2.26^{b} \pm 0.02$	$2.15^{a} \pm 0.01$	**5.03****
Head Elongation	$0.38^{b} \pm 0.0025$	$0.36^{a} \pm 0.0026$	**5.31****

*Data are presented as the mean ± SEM, n=512. a,b; Different superscripts indicate significant differences between fresh and cryopreserved semen within each parameter; *(P< 0.05),**(P< 0.01)*

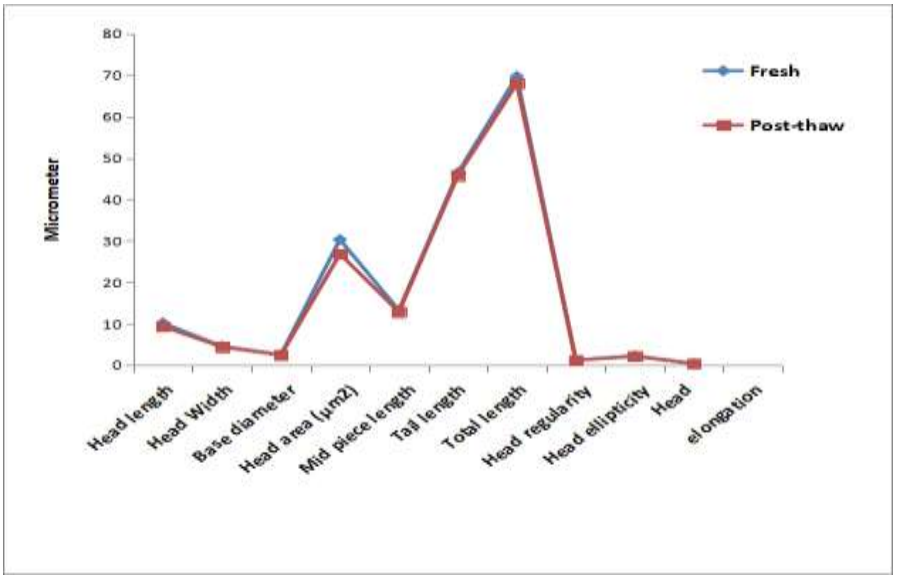

Fig. 1: Comparison of sperm morphometry (µm) in fresh and post thaw semen of Hariana bull

In agreement with our findings Gravance *et al* (1998) and Biswajit (2014) documented that bull sperm heads were significantly ($P < 0.01$) smaller in cryopreserved spermatozoa than the extended samples. The average values fall within the ranges reported by Biswajit (2014) and Ustuner *et al.* (2015). In agreement

DOI 10.5455/ijlr.20180827060644

with our findings Jiménez *et al.* (2006) reported that dimensions of goat sperm heads after cryopreservation were smaller than in fresh sperm and further stated that dead spermatozoa showed smaller heads than live sperm. The significant difference in the change in Width/Length appeared to be driven by decrease in length with a less noticeable decrease in width during cryopreservation (Gravance *et al.*, 2009) and this ratio is related to the fertility of bull. These findings suggest that changes in sperm head morphometry might indicate spermatozoa injury during cryopreservation.

In the case of sperm morphology, it has been exhibited that a decrease in the number of morphologically normal sperm in ejaculates leads to lower fertility (Hafez, 2000). Hence, the lower fertility of the cryopreserved semen samples may be a result of a decline in the number of normal sperm in these samples (Gravance *et al.*, 1997). The variation in the sperm measurements between fresh and frozen-thawed samples have been indirectly attributed to possible modifications of some of cell compartments (Gravance *et al*., 1998; Arruda *et al*., 2002), thus leading to the inference that changes in sperm head morphometry may stipulate which sperm will be frozen with greater success (Gravance *et al*., 1997; Kipper *et al*., 2017). Morphological alterations may be the key for the clarification of the differential fertilizing capacity and cryopreservation efficiency between samples (Soler *et al*., 2005). Bravo *et al.* (2014) observed seasonal variation in morphometric parameters with sperm size being larger in autumn and winter. Hidalgo *et al.* (2007) observed that the number of sperm morphometric parameters that had not changed after cryopreservation was increased in semen samples having high fertilizing ability after thawing. Therefore, it is rational to assume that in superior quality samples the sperm cryodamage was lower, and consequently the freeze-thawing effect on the sperm head morphometry was also lower, indicating better freezability. Thus, sperm head morphometric analysis might be included in the routine evaluation of semen used to assess the ability of semen samples to be successfully frozen. Ultrastructural damage to membranes due to cryopreservation disrupts them, predisposing sperm to gross morphologic defects, such as missing and abnormal acrosomes. It seems possible that modification of sperm membrane lipids interrupts the lipid-lipid and lipid-protein interactions required for normal membrane function which may play a key role in the inferior fertility of cryopreserved sperm.

Conclusion

In case of bulls, it is proved that variation in morphometry measurements may be associated with fertility potential and abnormal chromatin structure (Sailer *et al*., 1996). According to these findings, the changes in sperm head morphometry of frozen semen samples might be a outcome of chromatin over condensation or to acrosomal damage which causes diminished fertility. Our results were in line with earlier studies that used ASMA for bulls (Gravance *et al*., 1998; Gravance *et al*., 2009), stallions (Arruda *et al*., 2002) and red

 DOI 10.5455/ijlr.20180827060644

deers (Esteso *et al*., 2003) in which the sperm head dimensions of cryopreserved samples were significantly smaller than those of the fresh samples.

References

1. Andrabi, S.M.H. (2009). Factors affecting the quality of cryopreserved buffalo (*Bubalus bubalis*) bull spermatozoa. *Reproduction in Domestic Animals*. 44: 552-569.
2. Arruda, R.P., Ball, B.A., Gravance, C.G., Garcia, A.R. and Liu, I.K.M. (2002). Effects of extender and cryoprotectants on stallion sperm head morphometry. *Theriogenology.* 58: 253–256.
3. Biswajit, R. (2014). A com,parative study on sperm morphometry of crossbred and murrah buffalo bulls. *International Journal of Agricultural Sciences and Veterinary Medicine*. 2: 149-155.
4. Bravo, J.A., Montanero, J., Calero, R .and Roy, T.J. (2014). Influence of season and reproductive management on the morphometry of ram sperm head. *Small Ruminant Research*. 119: 114–119.
5. Esteso, M.C., Fernández-Santos, M.R., Soler, A.J. and Garde, J.J. (2003). Head dimensions of cryopreserved red deer spermatozoa are affected by thawing procedure. CryoLetters. 24: 261–268.
6. Francisco, A., Garcia, V., Joaquín, G., Carmen, M. and William, V.H. (2016). Importance of sperm morphology during sperm transport and fertilization in mammals. Asian *Journal of Andrology*. 18: 844–850.
7. Gangwar, C., Saxena, A, Patel, A., Singh, S.P. Yadav, S, Kumar, R. and Singh, V. (2018). Effect of reduced glutathione supplementation on cryopreservation induced sperm cryoinjuries in Murrah bull semen. *Animal Reproduction Science*. 192: 171-178.
8. Gangwar, C., Kharche, S.D. Ranjan, R. Kumar, S. Goel, A.K. Jindal, S.K. and Agarwal, S. K. (2015). Effect of vitamin C supplementation on freezability of Barbari buck semen. *Small Ruminant Research*. 129: 104–107.
9. Gangwar, C., Ranjan, R., Kumar, S., Kharche, S.D. Goel, A.K. Ramachandran, N. and Jindal, S.K. (2014). Use of chelating agent for optimum post thaw quality of buck semen. *Indian Journal of Animal Science. 84: 839–841*.
10. Gravance, C.G., Robertson, K.R., White, C. and Casey, P.J. (1997). The effects of cryo- preservation on the morphometric dimensions of caprine sperm heads. *Animal Reproduction Science*. 49: 37-43.
11. Gravance, C. G., Vishwanath, R. Pitt, C. Garner, D.L. and Casey, P.J. (1998). Effects of cryopreservation on bull sperm head morphometry. *Journal of Andrology*. 19: 704–709.
12. Gravance, C.G., Casey, M.E. and Casey, P.J. (2009). Pre-freeze bull sperm head morphometry related to post-thaw fertility. *Animal Reproduction Science*. 114: 81–88.
13. Hafez, E.S.E. (2000). Reproduction in farm animals. 7th Edn., P: 293-367.
14. Hancock, J.L. (1951). A staining techniques for the study of temperature shock in semen. *Nature* (London), 169: 323-326.
15. Hidalgo, M., Rodríguez, I. and Dorado, J.M. (2007). The effect of cryopreservation on sperm head morphometry in Florida male goat related to sperm freezability. *Animal Reproduction Science*. 100: 261–72.
16. Jiménez, M.F., Viudes-de-Castro, M.P., Moce, S.E., Silvestre, M.A., Gomez, E.A. and Vicente, J.S. (2006). Morphometric changes in goat sperm heads induced by cryopreservation. *Cryobiology*. 52: 295–304.
17. Kipper, B.H., Trevizan, J.T., Carreira, J.T., Carvalho, I.R. Mingoti, G.Z, Beletti, M.E. Perri, S.H.V., Franciscato, D.A, Pierucci, J.C. and Koivisto, M.B. (2017). Sperm morphometry and chromatin condensation in Nelore bulls of different ages and their effects on IVF. *Theriogenology*. 87: 154–160.
18. Ostermeier, G.C., Sargeant, G. A., Yandell, B. S. Evenson, D.P. and Parrish, J.J. (2001). Relationship of bull fertility to sperm nuclear shape. *Journal of Andrology*. 22: 595–603.
19. Pena, F.J., Saravia, F., García-Herreros, M., Nunez, I., Tapia, J.A., Johannisson, A., Wallgren, M. and Rodríguez-Martínez, H. (2005). Identification of sperm morphological subpopulations in two different portions of the boar ejaculate and its relation to post thaw quality. *Journal of Andrology*. 26: 716-723.

 DOI 10.5455/ijlr.20180827060644

20. Sailer, B.L., Jost, L.K. and Evenson, D.P. (1996). Bull sperm head morphometry related to abnormal chromatin structure and fertility. *Cytometry*. 24: 167–173.
21. Saravia, F., Núñez-Martínez, I., Morán, J.M. Soler, C., Muriel, A., Rodríguez-Martínez, H. and Peña, F.J. (2007). Differences in boar sperm head shape and dimensions recorded by computer-assisted sperm morphometry are not related to chromatin integrity. *Theriogenology*. 68: 196-203.
22. Thurston, L.M., Watson, P.F., Mileham, A.J. and Holt, W.V. (2001). Morphologically distinct sperm subpopulations defined by Fourier shape descriptors in fresh ejaculates correlate with variation in boar semen quality following cryopreservation. *Journal of Andrology*. 22: 382–94.
23. Ustuner, B., Zekariya, N., Selim A., Mehmet, B. T., Hakan S., Mustafa K. (2015). Effect of freezing rate on goat sperm morphology and DNA integrity. *Turk J Vet Anim. Sci*. 39: 110-114.
24. Zhang, B.R., Larsson, B. Lundeheim, N. and Rodriguez-Martinez, H. (1998). Sperm characteristics and zona pellucida binding in relation to field fertility of frozen thawed semen for dairy AI bulls. I*nternational Journal of Andrology*. 21: 207–16.

DOI 10.5455/ijlr.20180827060644