Indian Journal of Animal Sciences **88** (3): 311–313, March 2018/Short communication

Correlation of HOST to some conventional sperm quality parameters evaluated in frozen thawed semen of Frieswal bulls

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Received: 14 July 2017; Accepted: 18 October 2017

Key words: Frieswal bull, Hypo-osmotic swelling test, Semen quality, Thawed semen

Artificial insemination is a novel technique by which the germplasm of superior bulls are effectively used to improve livestock performance since the demand of good quality semen is increasing. During cryopreservation, the sperm cells are damaged (Kumar *et al.* 2016). Routinely, many lab tests such as incubation test, acrosome integrity, HOS test and motility are taken into consideration to evaluate sperm quality. Although no single measurement of semen quality is considered reliable for predicting fertility (Faulkner and Pineda 1980), one such quick and easy tool of evaluating sperm membrane integrity by Jeyendran *et al*. (1984) has proved to be fundamentally important in the fertilization process (Rota *et al*. 2000, Lodhi *et al*. 2008). A positive correlation was reported between the results of the HOS test and the non-return rate of female animals (Correa *et al.* 1997), which potentially makes it one of the most appropriate and simple methods for semen quality evaluation (Padrik *et al.* 2012). In order to produce frozen semen of uniform quality, a Minimum Standard Protocol (MSP) for semen production has been developed in consultation with experts from Bharatia Agro-Industries Foundation (BAIF), National Dairy Development Board (NDDB), National Dairy Research Institute (NDRI) and Central Frozen Semen Production and Training Institute (CFSP & TI) and the same has been made effective from 2004 (Department of Animal Husbandry, Dairying and Fisheries). Keeping in view the above facts, the study was undertaken to find out the relationship of HOS test to other sperm quality parameters (post thaw motility, incubation test and acrosome integrity) in frozen semen straws and to validate the viability of HOS test to evaluate sperm quality.

Frozen semen straws (119) maintained at Bull Rearing Unit, Central Institute for Research on Cattle, Meerut were assessed for the post thaw quality. According the Central Monitoring Units (CMU) for semen stations, the ejaculates with motility ≥70% and concentration ≥500 millions/ml were processed after dilution in egg yolk tris buffer citrate and packed in 0.25 ml straws for cryopreservation. The acceptable limit of post thaw quality is 50% for motility, 10% drop in motility every 30 min in incubation test, acrosome integrity above 65% and HOS test above 40%. After cryopreservation, the following parameters were subjectively evaluated as per the methods described. In order to estimate the post thaw motility, frozen semen samples were thawed at 37°C for 60 sec. A drop of sample was placed on a slide with a cover slip on a warm stage and subjectively assessed using phase contrast microscope at 400× magnification. The incubation test was assessed after incubating the semen straws at 37°C for 1h and the motility was subjectively assessed. Acrosomal integrity (% normal) based on acrosomal damage was studied by Giemsa stain (Watson 1975). Fig. 1 represents the acrosome integrity and HOS positive sperm cells under microscope. Hypo-osmotic swelling test was carried out as per Jeyendran *et al*. (1984). A drop of diluted semen was placed on a clean sterilized dry glass slide and covered with a cover slip. A total of 200 spermatozoa were counted in different fields at 400× under phase contrast microscope and percentage of spermatozoa positive to HOS test (having coiled tails) was determined. Further, two groups were made according to the HOS reactions and compared for the quality parameters. One group consisted of HOS reaction above 40% and another group below 40% respectively.

The Pearson linear correlation method (Steel and Torrie

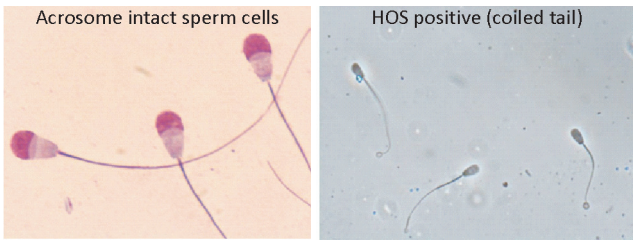

Fig.1. Acrosome intact and HOS positive (tightly coiled sperm tail) sperm cells seen under microscope

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Table 1. Correlation between hypo-osmotic swelling test (HOST) with acrosome integrity, post thaw motility and incubation test

	HOST	Post thaw motility	Incubation test (one hour)	Acrosome
Host	1			
PTM	0.132	1		
IT (60)	0.106	0.522**	1	
Acrosome	0.308**	0.298**	0.200*	1

*P<0.05 level and ** P<0.01 level

that were routinely used for semen collection.

Therefore, it can be inferred that HOS test may be a valuable guide for routine semen quality evaluation on daily basis to acquire a quick quality result.

SUMMARY

In this study, hypo-osmotic swelling test (HOST) was used to correlate with other sperm quality parameters such as post thaw motility, incubation test and acrosome integrity. In present investigation, the HOS test had positive significant correlation to acrosome integrity. Also the

Table 2. Quality semen parameters in hypo-osmotic swelling test reactive groups (HOST>40% and HOST<40%)

Group	Neat semen			Post thaw semen quality parameters			
	Volume (ml)**	Motility (%)	Concentration of neat (10^6/ml)**	Post thaw motility (%)**	Incubation test (%)	Acrosome integrity (%)**	Host (%)**
HOST>40% (n=92)	5.86±0.18	71.73±0.39	1264.70±54.00	55.32±0.64	38.91±0.90	67.32±0.63	52.37±0.85
HOST<40% (n=27)	4.15±0.25	71.48±0.68	924.03±43.04	51.48±1.02	35.37±2.08	60.98±1.54	31.12±1.24

**P<0.01 level.

1980) was used to correlate HOS test to other sperm parameters (acrosome integrity, post thaw motility and incubation test) and t- test was applied for significance in the two HOS reacted groups (>40%; <40%). Difference were considered significant when P≤0.05.

The semen of bulls that were used for cryopreservation had the overall neat semen quality parameters as per the MSP guidelines (5.47±0.34 ml, 71.68±0.72% and 1187.41±95.17 million/ml for volume, motility and concentration respectively). Simultaneously, post thaw semen quality was 54.45±1.19%, 38.10±1.78%, 65.89±1.36% and 47.55±2.28% for motility, incubation test, acrosome integrity and HOS positive respectively. However, the post thaw motility and HOS test reported by Mandal *et al*. (2008) in Frieswal bull was 46.24±0.46 and 33.67±1.25% respectively. The results (Table 1, 2) showed a positive correlation between HOS test and post thaw motility (r=0.132), incubation test (r=0.106) and acrosome integrity (r=0.308; P<0.01). Similar findings were corroborated by Lodhi *et al.* (2008) in Sahiwal bull and Nilli Ravi buffaloes. The mean post thaw motility (PTM) at 0 min was 54.45±1.19 and after incubating it for 60 minutes at 37°C it was 38.10±1.78% respectively. These findings were in close agreement to Kedai *et al.* (2013) in Tharpakar bulls. When, the post thaw HOS test was above 40% (52.37±0.85%), the motility and acrosome integrity was highly significant (P<0.01) to those samples having a HOS test below 40% (31.12±1.24%). The finding has been well supported by Zubair *et al.* (2013) in Sahiwal, Fresian and Crossbred bulls. However, HOS test was significantly (P<0.01) correlated to acrosome integrity which is also a stable parameter of sperm function (Henkel *et al.* 1994) and fertility in males (Suri 2005) therefore, good HOS test reaction also means to have a good fertility. However, the post thaw semen samples tested were from the good bulls samples which had post thaw HOS test above 40% had high significant levels of post thaw motility and acrosome integrity when compared to the samples having poor HOS test response below 40%. It is likely an easy and inexpensive test to differentiate between good and poor sperms hence may be routinely used in semen stations.

ACKNOWLEDGEMENT

The authors are thankful to Director, ICAR-Central Institute for Research on Cattle, Meerut, India for providing necessary facilities for conducting the study. We also acknowledge Director, Military Farms and Frieswal Project for providing necessary information and data.

REFERENCES

Correa Jr, Heerche Jr G and Zavos P. 1997. Sperm membrane functional integrity and response of frozen thawed bovine spermatozoa during the hypoosmotic swelling test incubation at varying temperatures. *Theriogenology* **47**: 715–21.

Faulkner L C and Pineda M H. 1980. Artificial insemination. *Veterinary Endocrinology and Reproduction.* 3rd edn, pp. 330–336. (Ed) Donald M C. Lea and Febiger, Philadelphia.

Henkel R, Muller C, Miska W, Gips H and Schill W. 1993. Determination of the acrosome reaction in human spermatozoa is predictive of fertilization *in vitro*. *Human Reproduction* **8**: 2128–32.

Jeyendran R S, Van Der Ven H H, Perez-Pelaez M, Grabo B G and Zanaveld L Z D. 1984. Development of an assay to assess the functional integrity of human sperm membrane and its relationship to other sperm characteristics. *Journal of Reproduction and Fertility* **70**: 219–28.

Kedia N K, Tiwari R P, Mishra G K, Awasthi M K and Sahu S B. 2013. Effect of post thaw incubation on quality of frozen Tharparkar bull semen. *Indian Journal of Animal Reproduction* **34**(2): 9–12.

Kumar D, Kumar P, Singh P, Yadav S P and Yadav P S. 2016. Assessment of sperm damages during different stages of

cryopreservation in water buffalo by florescent probes. *Cytotechnology* **68**: 451–58.

Lodhi L A, Zubair M, Qureshi Z I, Ahmad I and Jamil H. 2008. Correlation between hypo-osmotic swelling test and various conventional semen evaluation parameters in fresh Nili-Ravi buffalo and Sahiwal cow bull semen. *Pakistan Veterinary Journal* **28**(4): 186–88.

Mandal D K, Tyagi S and Kumar M. 2008. Effect of types of prepuce on semen production performance of Frieswal bulls. *Indian Journal of Animal Sciences* **78**(11): 1259–60.

Padrik P, Hallap T, Kaart T, Bulitko T and Jaakma U. 2012. Relationships between the results of hypo-osmotic swelling tests, sperm motility, and fertility in Estonian Holstein dairy bulls. *Czech Journal of Animal Science* **57**: 490–97.

Rota A, Penzo N, Vincenti L and Mantovani R. 2000. Hypo osmotic swelling as a screening assay for testing *in vitro* fertility of bovine spermatozoa. *Theriogenology* **53**(7): 1415–20.

Steel R G D and Torrie J H. 1980. *Principles and Procedures of Statistics- A Biometrical Approach*. 2nd edn. McGraw Hill Inter. Book Co. Tokyo, Japan.

Suri A. 2005. Contraceptive vaccines targeting sperm. *Expert Opinion in Biological Therapy* **5**: 381–92.

Watson P F. 1975. Use of a Giemsa stain to detect changes in the acrosomes of frozen Ram spermatozoa. *Veterinary Record* **97**: 12–15.

Zubair M, Laeeq A L, Ejaz A and Ghulam M. 2013. Hypo-osmotic swelling test as screening for evaluation of semen of bull. *Journal of Entomology and Zoology Studies* **1**(6): 124–28.