Explor. Anim. Med. Res.,
Vol.3, Issue - 2, 2013, p. 159-164

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

A COMPARATIVE STUDY WITH MURRAH BUFFALO AND INDIGENOUS GIR SPERMATOZOA TO HYPO-OSMOTIC SWELLING TEST

Sumit Chowdhury*, Srinibas Das, Tapas Gupta, Debdulal Sana and Subhas Bose[1]

ABSTRACT: Hypo-Osmotic Swelling Test (HOST) is a simple but authentic test to measure the integrity of sperm membrane. The present study was undertaken to evaluate the HOST value in Murrah buffalo and indigenous Gir bull in 150 mosm/l tri-sodium citrate and D-fructose HOST solution. Murrah buffalo was having more HOST (49±0.39 %) reacted spermatozoa than indigenous Gir (42±0.57 %) bulls suggesting lesser membrane damage during cryo-preservation and higher fertility rate in Murrah buffalo than Gir cattle.

Key words: HOST, PTM, Gir, Murrah, Spermatozoa.

INTRODUCTION

Artificial Insemination has played a significant role in dairy industry through production and preservation of semen from elite bulls. However, the prediction of potential fertility bull is always obtained by evaluation of different parameters of semen. The structural integrity of spermatozoa is an important parameter for semen evaluation (Neild *et al.* 1999). Post-thaw viability and fertility of cryopreserved sperm are often reduced due to accumulated cellular damage during the cryo-preservation phases (Muiño *et al*. 2008). Decrease in temperature, cold shock, and intracellular ice formation can affect sperm plasma membrane, acrosomal and mitochondrial membrane integrity (Thomas *et al.* 1998, Defoin *et al.* 2008) and cause the loss of intracellular components (Graham and Mocé 2005), which can initiate cell death. Sperm membrane integrity can be evaluated by several methods like, light or fluorescent microscopy combined with vital stains (Brito *et al.* 2003), and flow cytometry (Januskauskas *et al.* 2003). Jeyendran *et al.* (1984) developed a hypo osmotic swelling test (HOST) to evaluate sperm membrane function of human spermatozoa. HOST is used to evaluate the sperm membrane integrity in bovines (Rota *et al.* 2000), equine (Neild *et al.* 2000), canine (Rodriguez-Gil *et al.* 1994) and porcine (Perez-Llano *et al*. 2001). The present study was envisaged to compare between percent HOST positive sperm of Murrah buffalo and Gir bull semen after cryo-preservation and to find out the breed average values of HOST, a directly fertility related test to evaluate cryo-preserved semen.

Frozen Semen Bull Station (Paschim Banga Go-Sampad Bikash Sanstha),Composite State Animal Husbandry Farm, Salbini, Paschim Medinipur-721147, West Bengal, India.
[1]Chief Executive Officer, Paschim Banga Go-Sampad Bikash Sanstha, Kolkata -98, West Bengal, India.
**Corresponding author.*

METERIAL AND METHODS

Preparation of Frozen Semen Straw (FSS):

Ten ejaculates from each Murrah buffaloes and Gir bulls of different days over a period of six months were used in this study. Bulls were kept at the Frozen Semen Bull Station, Salboni, Paschim Medinipur, West Bengal. Semen was collected using Artificial Vagina (IMV, France). Neat semen of each and every ejaculate was evaluated in volume, concentration and motility. The percentage motility less than 70% were discarded. The volume above 2 ml of neat semen was accepted. The concentration was measured in photometer (Accucell, IMV, France) at 540 nm wavelength. The concentration above 500 million/ml of neat semen was used for further processing. Diluted semen was prepared by mixing neat semen with Tris-egg yolk citrate dilutor. The filling and sealing of straw of 0.25 ml volume was performed in IS-4 (Instrument Synchronization) machine (IMV) with software called System Management in Integrated Laboratory Environment (SMILE). Freezing of semen was done using conventional method *viz.* equilibration at 4^0C for 4 hr in Gir, 3 hr in buffalo, followed by gradual drop in temperature from 4^0C to -140^0C in 9 min in Bio-freezer backed up by software and finally shifting the straws in liquid nitrogen at -196^0C.

Post Thawed Motility (PTM) and Incubation Test:

The stored FSS was removed from LN_2 after 24 hrs incubation. The motility was analyzed in phase contrast microscope at 200X (10 X 20) magnification. Firstly, the straw was thawed vertically in 37^0C in water bath for 30 sec. They were then removed from the water bath, dried with napkin and the semen was poured in a sugar tube kept at 37^0C in a water bath. The progressive motility above 50% cut off value was chosen to keep the straw for further quality test. The FSS passed in PTM was further kept in the water bath up to 2 hrs. The motility was checked every half an hour interval and recorded.

Hypo-osmotic Swelling Test:

During the HOS test, the biochemically active spermatozoa are exposed to hypo osmotic stress. To establish equilibrium between the fluid compartment within the spermatozoon and extra cellular environment, sperm cell will undergo swelling due to influx of water. The plasma membrane surrounding the tail fibres appear to be more loosely attached than the membrane surrounding the head, so that the tail region shows the swelling more clearly.

Preparation of Hypo-osmotic solutions:

These were prepared by dissolving 0.735 gm tri-sodium citrate di-hydrate (Merck, Germany) and 1.351 gm D- fructose (Merck) in 100ml of Milli-Q water (Millipore) to attain the 150mosm/l. The solution was stored at 4^0C for further use.

HOS Test:

1.0 ml of HOST solution was pre warmed at 37^0C temperature in water bath. 0.1 ml of thawed semen was added and mixed gently in HOST solution with micro pipette. The mixture was subjected to incubation for 1 hr at 37^0C. Then one drop of 3mm size of that solution was put on a clean glass slide and a smear was made. The smear was then dried in air and stained with Rose Bengal for 10 minute. Same way 0.1 ml semen was tested in 0.9% NaCl solution. The stained slide was washed in running tap water and air dried. Total 100 coiled spermatozoa were counted in 400X magnification in Differential

Table 1. Values of different parameters of neat semen and frozen semen of individual samples of Gir cattle and Murrah buffalo.

Breed	Sample No	Volume (ML)	ANALYSIS OF NEAT SEMEN		ANALYSIS OF FROZEN SEMEN (%)					
			Concentration (Million/Ml)	Motility (%)	PTM	INC 30M	IND 60M	IND 90M	INC 120M	HR
GIR	1	8.7	820	75	55	45	35	25	15	40
	2	6	607	75	55	45	35	25	15	42
	3	7.5	650	75	55	45	35	25	14	45
	4	11.5	739	75	55	45	35	25	15	43
	5	7.1	1024	75	55	45	35	25	15	41
	6	4.1	822	75	60	50	40	30	20	45
	7	3.5	507	75	55	45	35	25	15	40
	8	4	861	75	55	45	35	25	15	42
	9	3	503	75	55	45	35	25	15	41
	10	8.5	830	80	60	50	40	30	20	42
MURRAH	1	3	571	75	55	45	35	25	15	49
	2	3	533	75	55	45	35	25	15	48
	3	3	600	80	55	45	35	25	15	48
	4	5.4	505	75	60	50	40	30	20	50
	5	5.6	510	75	55	45	35	25	15	51
	6	3	510	75	60	50	40	30	20	48
	7	3.2	1066	80	60	50	40	30	20	51
	8	2.5	833	75	55	45	35	25	15	48
	9	3.2	828	80	55	45	35	25	15	49
	10	4.5	528	75	55	45	35	25	15	48

(PTM - Post thaw motility; INC 30M - Incubation at 30 MINUTE; INC 60M - Incubation at 60 minute; INC 90M - Incubation at 90 minute; INC 120M - Incubation at 120 minute; HR – Host reacted)

Interference Contrast (DIC) Olympus microscope (BX51).

Statistical analysis of data was done as per standard procedure expressing the observations as means ± SE.

RESULT AND DISCUSSION

The volume, motility and concentration of neat semen checked in our laboratory instruments were given in Table 1. This indicated that the volume of all neat semen irrespective of species were above 2 ml, concentration above 500 million/ml of fresh semen and progressive motility above 70%, envisaged all semen were in good condition for further processing and preservation. It has been observed that the volume of semen per ejaculation was higher in case of indigenous Gir (6.39±0.87 ml) than Murrah buffalo (3.64±0.35 ml).

The PTM value of all FSS analyzed after 24 hrs of preservation shown (Table1) that all values were above 50%. The qualifying FSS was subjected for incubation test at 37^0C for 2 hr. The motility drops in each half an hour were shown in Table1. The FSS qualified in both PTM and incubation was used in HOST reaction. The values of HOST reacted coiled and swollen tailed positive sperm percentage is given in Table 1.

The aim of the study was to focus on comparison of the HOST reaction in Murrah Buffalo and indigenous Gir bull frozen spermatozoa. Several authors have validated the suitability of the HOST for assessing the quality

Table 2. Breed wise mean values of volume and concentration of neat semen and post thaw motility, incubation test and hypo-osmotic swelling test of frozen semen of Gir and Murrah.

BREED	PARTICULARS	VOLUME OF NEAT SEMEN OF AN EJACULATION (ML)	CONCENTRATION OF NEAT SEMEN (MILLION/ML)	POST THAW MOTILITY (%)	INCUBATION AT 30 MINUTE (%)	INCUBATION AT 60 MINUTE (%)	INCUBATION AT 90 MINUTE (%)	INCUBATION AT 120 MINUTE (%)	% HOST REACTED
GIR	**MEAN**	**6.39**	**736**	**56**	**46**	**36**	**26**	**16**	**42**
	STDEM	0.87	53	0.67	0.67	0.67	0.67	0.67	0.57
MURRAH	**MEAN**	**3.64**	**648**	**57**	**47**	**37**	**27**	**17**	**49**
	STDEM	0.35	61	0.76	0.76	0.76	0.76	0.76	0.39

of human semen (Moskovtsev *et al.* 2005, Cincik *et al*. 2007) and also the semen of various domestic animals including cattle (Correa and Zavos 1994), horses (Neild *et al*. 1999) and pigs (Gadea *et al*. 1998). As the test gives a consistent estimate of the percentage of spermatozoa with a physiologically active membrane, it predicts the fertilizing capacity of spermatozoa in animals (Brito *et al.* 2003). In bovine Rovell and Mrode (1994) reported a high correlation (0.79) between HOS test results and fertility (expressed as non -return rate) when the bull effect was removed. Januskauskas *et al*. (1996) have observed associations between ATP content and sperm membrane integrity, assessed using fluorophore probes. Zuge *et al.* (2008) have reported a high positive correlation between the proportion of sperm cells with high mitochondrial activity and that with intact membranes, determined using the HOST.

As depicted in Table 2 percent HOST reacted spermatozoa were higher in case of Murrah Buffalo (49±0.39 %) than indigenous Gir cattle (42±0.57 %). As reported by several researchers mentioned earlier HOS test is indicative towards male fertility as well as membrane damage during cryopreservation. Present study indicates higher permeability of sperm membrane in HOST solution of Murrah buffalo than indigenous Gir cattle. It is also suggestive towards lesser membrane damage during cryopreservation and higher fertility rate in Murrah than Gir. On contrary, Lodhi *et al.* (2008) reported an almost similar HOST value in Nili-Ravi Buffalo and Sahiwal Bull. Padrik *et al.* (2012) found that good fertility of spermatozoa tested in different HOST solutions in decreased osmolarity in Estonian Holstein bulls. Further investigation of osmotic resistance of spermatozoa with decreased different osmotic pressure in both Murrah and Gir requires to be compared for better interpretation of the present findings.

REFERENCES

Brito LFC, Barth AD, Bilodeau-Goeseels S, Panich PL and Kastelic JP.(2003). Comparison of methods to evaluate the plasmalemma of bovine sperm and their relationship with *in vitro* fertilization rate. *Theriogenology*. 60: 1539- 1551.

Cincik M, Ergur AR, Tutuncu L, Muhcu M, Kilic M, Balaban B and Urman B.(2007). Combination of hypoosmotic swelling/eosin Y test for sperm membrane integrity evaluation: correlations with other sperm parameters to predict ICSI cycles. *Archives Androl*. 53: 25-28.

Correa JR and Zavos PM.(1994). The hypo - osmotic swelling test: its employment as an assay to evaluate the functional integrity of the frozen-thawed bovine sperm membrane. *Theriogenology*. 42: 351-360.

Defoin L, Granados A and Donnay I.(2008). Analyzing motility parameters on fresh bull semen could help to predict resistance to freezing: a preliminary study. *Reprod. Domestic Anim.* 43: 606-611.

Gadea J, Matas C and Lucas X. (1998). Prediction of porcine semen fertility by homologous in vitro penetration (hIVP) assay. *Anim. Reprod. Sci*. 54: 95-108.

Graham JK and Mocé E. (2005). Fertility evaluation of frozen/thawed semen. *Theriogenology.* 64: 429-504.

Januskauskas A, Söderquist L, Håård M.G, HåårdM.Ch, Lundeheim N and Rodriquez-Martinez H.(1996). Influence of sperm number per straw on the post-thawsperm viability and fertility of Swedish Red and White A.I. bulls. *Acta Veterinaria Scandinavica*. 34: 461-470.

Januskauskas A, Johannisson A and Rodriques-Martinez H.(2003). Subtle membrane changes in cryopreserved bull semen in relation with sperm viability, chromatin structure, and field fertility. *Theriogenology*. 60: 743-758.

Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG and Zaneveld LJD.(1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J. Reprod.Fertility*. 70: 219-225.

Lodhi LA, Zubair M, Qureshi ZI, 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 Vet. J.* 28(4): 186-188.

Moskovtsev SI, Wilis J, Azad A and Mullen JBM.(2005). Sperm DNA integrity: correlation with sperm plasma membrane integrity in semen evaluated for male infertility. *Archives Androl*. 5: 33-40.

Muiño R, Tamargo C, Hidalgo CO and Peña AI.(2008). Identification of sperm subpopulations with defined motility characteristics in ejaculates from Holstein bulls: effects of cryopreservation and between-bull variation. *Anim. Reprod. Sci*. 109: 27-39.

Neild D, Chaves G, Flores M, Mora M, Beconi M and Agüero A.(1999). Hypo-osmotic test in equine spermatozoa. *Theriogenology*. 51: 721-727.

Neild D, Chaves G, Flores M, Miragaya MH, Gonzalez A and Aguero A.(2000). The hypo-osmotic swelling test and its relationship to fertility in the stallion. *Andrologia*. 32(6): 351-355

Padrik P, Hallap T, Kaart T, Bulitk T and Jaakma U. (2012). Relationships between the results of hypo-osmotic swelling tests, sperm motility and fertility in Estonian Holstein dairy bulls. *Czech J. Anim. Sci.* 57(10): 490–497.

Pérez-Llano B, Lorenzo JL, Yenes P, Trejo A and García-Casado P. (2001). A short hypo osmotic swelling test for the prediction of boar sperm fertility. *Theriogenology*. 56: 387-398.

Rodriguez-Gil JE, Monserrat A and Rigau T. (1994). Effects of hypo-osmotic incubation on acrosome and tail structure of canine spermatozoa. *Theriogenology*. 42(5): 815-829.

Rota A, Penzo N, Vincenti L and Mantovani R.(2000). Hypo-osmotic swelling (HOS) as a screening assay fortesting *in vitro* fertility of bovine spermatozoa. *Theriogenology.* 53: 1415-1420.

Rovell SG and Mrode RA. (1994). An osmotic resistance test for bovine semen. *Anim. Reprod. Sci.* 36: 77-86.

Thomas CA, Garner DL, De Jarnette JM and Marshall CE.(1998). Effect of cryopreservation on bovine sperm organelle function and viability as determined by flow cytometry. *Biol. Reprod*. 58: 786-793.

Zuge RM, Bertolla RP, Nichi TBS, Cortada CNM and Bols PEJ. (2008). Correlation between bovine sperm membrane integrity and mitochondrial activity in *Bos taurus* bulls. In*: Proc. 16th International Congress on Animal Reproduction.* Budapest. Hungary. 172.

***Cite the article as:** 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(2): 159-164.