EFFECT OF CYSTEINE HYDROCHLORIDE ON POST THAW VIABILITY AND ACROSOMAL INTEGRITY OF JERSEY BULL SEMEN[#]

Anavil Bhardwaz[1], A. K. Pandey[2], Utsav Sharma , W. A. A. Razzaque, S. K. Gupta[3], Sharad Kumar[4] and Sudershan Kumar[5]

Division of Veterinary Gynaecology and Obstetrics

Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R. S. Pura, Jammu-181 102, Jammu and Kashmir, India

Received: 03.02.2016
Accepted: 19.09.2016

ABSTRACT

A study was undertaken to assess the efficacy of cysteine hydrochloride concentrations on post-thaw progressive motility, live sperm, acrosome integrity and sperm abnormalities following freezing and thawing in Jersey bulls semen. Semen was collected from 3 purebred Jersey bulls twice a week and 18 collections (6 collections per bull) were utilized in this study. After collection and primary evaluation, samples were divided into four fractions and were diluted in egg yolk tris glycerol extender with addition of cysteine @ 10, 12 or 15 mM (CYS-10, CYS-12 and CYS-15, respectively) and without cysteine (EYTG-C) which acted as control. Filling and sealing of diluted semen samples in PVA straws, equilibration at 4°C for 4 h, freezing in programmable bio-freezer and plunging of straws into liquid nitrogen were followed as per standard procedures. After thawing at 37°C for 30 sec, semen characteristics such as progressive motility, live sperm percentage and acrosome integrity were significantly higher whereas sperm abnormalities were significantly lower in samples containing cysteine @ 10 mM over the control and other dilutors. The results revealed that addition of cysteine @ 10 mM as an additive to semen in tris egg yolk extender, significantly improves the post-thaw semen characteristics, whereas adverse effect of cysteine was evident in its higher concentrations i.e. 12 mM and 15 mM.

Key words: Cysteine, post-thaw semen quality, tris, bull semen, cryopreservation

Introduction

Crossbreeding of non-descript cows with high yielding exotic bulls is a well adopted technique for improvement of genetic merit and production potential of future progeny. Long term storage of semen facilitates easy transport over distances and enables maximized use of a bull, even after the sire's death. Freezing and thawing exerts physiological as well as chemical stress on the sperm membrane (Chatterjee *et al.,* 2001), associated with an oxidative stress induced by free radical (Salvador *et al.,* 2006). Dilution of semen reduces the anti-oxidant reserves of seminal plasma, making the spermatozoa more susceptible to cryodamage (Muzafer *et al*., 2012). Therefore, it becomes all the more necessary to incorporate anti-oxidants to semen extenders as protective agents.

Garner *et al.* (1994) reported that post thaw sperm quality and fertility from a routine sperm function assay would be beneficial in predicting bulls fertility. The combined assessment of acrosome integrity and semen motility had a significant effect on fertility (Kjaestad *et al.,* 1993), thus is a reliable basis for post thaw estimation of semen quality.

Cysteine is a thiol compounds used in intracellular glutathione biosynthesis (Meister and Tate, 1976). Addition of cysteine to the semen dilutors have improved post thaw semen quality in different species (Uysal and Bucak, 2007; Perumal, 2008; Beheshti *et al.,* 2011). Thus, the present work was undertaken to assess the beneficial effects of Cysteine, on post-thaw semen quality following freezing and thawing in Jersey bulls.

Materials and Methods

Three adult, sexually mature Jersey bulls maintained at frozen semen bull station, Hakkal, Jammu were selected for this study. The bulls were maintained under scientific and uniform conditions of feeding and management throughout the experimental period. Semen was collected twice a week between 7.00 AM and 8.00 AM using artificial vagina following standard protocol using a male partner as dummy. On each collection day, 2 ejaculates were collected at an interval of 15-20 min and the semen was kept in a water bath at 37°C till further processing. Total 18 collections from 3 bulls (6 collections/bull) were utilized in this study.

After initial evaluation, each semen sample based on at least 75% initial motility was divided into four equal fractions; one fraction was diluted in egg yolk tris glycerol extender without any additive which acted as control (EYTG-C), second fraction was diluted in egg yolk tris glycerol with addition of cysteine @ 10 mM (CYS-10), third fraction was diluted in egg yolk tris glycerol with addition of cysteine @ 12 mM (CYS-12) and fourth fraction was diluted in egg yolk tris glycerol with addition of cysteine @ 15 mM (CYS-15). The semen was diluted in such a way that it contained 80 million spermatozoa/ml post dilution. The diluted semen was filled in 0.25 ml poly vinyl chloride straws using an automatic filling and sealing machine. After equilibration for 4 hrs, sealed straws were frozen using programmable bio-freezer where the temperature was brought down from 4 to -140°C in 7 min. The straws were transferred to pre-cooled plastic goblets and plunged into liquid nitrogen. After 10-15 days of preservation in liquid nitrogen, at least 2

[1#]Part of M.V.Sc. Thesis submitted by first author; [2]Corresponding author email: anil_p8@yahoomail.com; [3]Division of VMD, F.V.Sc. & A.H., [4]Division of TVCC, F.V.Sc. & A.H.; [5]Department of VGO, College of Veterinary Sciences & A.H. NDVSU, Rasalpura Mhow

Table 1: Progressive motility, live sperm and acrosome integrity in frozen thawed Jersey bull semen supplemented with cysteine hydrochloride (Mean ± SE)

TYPE OF DILUENT	Progressive motility (%)	Live sperm (%)	Acrosome integrity (%)	Sperm abnormalities(%)
EYTG-C (Control)	53.88±0.43^{c}	54.38±0.49^{c}	74.83±0.44^{c}	10.38±0.28^{b}
CYS-10	63.92±0.44^{d}	64.67±0.49^{d}	78.17±0.45^{d}	9.43±0.28^{a}
CYS-12	50.05±0.43^{b}	50.83±0.49^{b}	69.83±0.44^{b}	11.05±0.28^{b}
CYS- 15	40.05±0.43^{a}	40.83±0.49^{a}	59.83±0.44^{a}	22.11±0.28^{c}

Means bearing different superscripts with in a row differ significantly (P<0.05)

straws from each bull in each collection were thawed at 37°C for 30 sec and evaluated for various parameters such as progressive motility, livability, sperm abnormality and acrosomal integrity. The data recorded was analyzed as per the procedure described by Snedecor and Cochran (1994).

Results and Discussion

The effect of addition of cysteine hydrochloride to tris egg yolk extender on post-thaw semen progressive motility percentage, live percentage, intact acrosome percentage and total sperm abnormalities are presented in Table 1. In the present study, the post-thaw progressive motility, livability and acrosome integrity were significantly higher (P<0.05) and sperm abnormalities were significantly lower (P<0.05) in extenders containing cysteine hydrochloride @ 10 mM compared to control and other cysteine diluents. However, adverse effect of cysteine is evident at higher concentration dilutors. The present findings authenticate earlier reports on supplementation of antioxidants for improving the viability and motility of liquid storage or cryopreserved sperm cells (Baumber *et al.,* 2000; Maxwell and Stojanov, 1996; Perumal *et al*., 2011). Beneficial effect of cysteine might be due to its free radical scavenging property and reduction in lipid peroxidation of sperm plasma membranes.

Freezing-thawing process produces physical and chemical stress on the sperm cell membrane which in turn reduces sperm viability and fertilizing ability (Uysal and Bucak, 2007). The cold shock exerted on sperm cells during freezing to thawing process is associated with oxidative stress induced by free radicals (Sanocka and Kurpisz, 2004; Salvador *et al*., 2006).

Protection of sperm motility, viability and stabilization of sperm cells membrane during cryopreservation are of prime importance. Perusal of results indicates beneficial effect of cysteine @10 mM on sperm survival; where by increasing concentration of cysteine (12 mM and 15 mM) had adverse effect during the freezing thawing process. Similar results were reported by Uysal and Bucak (2007) by addition of different concentration (5 mM, 10 mM and 20 mM) of cysteine in tris based extender and better post thaw motilty of ram semen by addition of cysteine @ 10 mM. Funahashi and Sano (2005) and Szczesniak-Fabianczyk *et al.* (2003) reported that a semen extender with cysteine improved the viability, chromatin structure and membrane integrity of boar sperm cells during liquid preservation. Moreover, in horses (Baumber *et al.,* 2000) and in rams (Sarlos *et al*., 2002) there was no effect of Cysteine hydrochloride on the acrossomal membrane integrity. In man, semen showing high concentration of thiols is considered subfertile (Ebisch *et al.*, 2006).

Acknowledgements

The authors are thankful to the Dean, F.V.Sc. & A.H., SKUAST-J and the authorities of J & K Livestock Development Board, Department of A.H.D., Jammu for providing facilities for conducting present research work.

References

Baumber, J. *et al.* (2000) *J. Androl.* **21**: 895-902.
Beheshti. R. *et al.* (2011) *Adv. Environ. Biol.* **5:**1260-1263.
Chatterjee, S. *et al.* (2001) *Mol. Reprod. Dev.* **60**:498-506.
Ebisch, I. M. *et al.* (2006) *Hum. Reprod.* **21**: 1725-1733.
Funahashi, H. and Sano, T. (2005) *Theriogenol.* **63:** 1605-1616.
Garner, D. L. *et al.* (1994) *J. Androl.* **15**:620-629.
Kjaestad, H. *et al.* (1993) *Acta. Vet. Scand*. **34**:299-303.
Maxwell, W. M. and Stojanov, T. (1996) *Reprod. Fertil. Dev.* **8**:1013-1020.
Meister, A. and Tate, S. S. (1976) *Annu. Rev. Biochem.* **45**: 559-604.
Muzafer, A. B. *et al.* (2012) *Indian J. Ani. Sci.* **82**: 457-460.
Perumal, P. (2008) *Reduced glutathione and cysteine hydrochloride on crossbred bull semen*. M. V. Sc. Thesis, submitted to Orissa Agriculture University, Bhubaneswar, India.
Perumal, P. *et al.* (2011) *Reprod. Domest. Ani.* **46**(**4**):636-641.
Salvador, I. *et al.* (2006) *Theriogenol.* **66**: 974-981.
Sanocka, D. and Kurpisz, M. (2004) *Reprod. Biol. Endocrinol.* **2**:12-18.
Sarlos, P. *et al.* (2002) *Acta Vet. Hung*. **50:** 235-245.
Snedecor, G.W. and Cochran, W. G. (1994) *Statistical Methods***.** 8th ed., Oxford and IBH, New Delhi, India.
Szczesniak-Fabianczyk, B. *et al.* (2003) *Reprod. Biol.* **3**: 81-87.
Uysal, O. and Bucak, M.N. (2007) *Acta Vet. Brno*. **76**: 383-390.