Cysteine Hydrochloride on Post-thawed Seminal Characters of Jersey Crossbred Bull

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Abstract

The role of cysteine hydrochloride in improving the post-thawed seminal characters in good and poor freezable Jersey crossbred bull semen was studied and compared with the control group. Two test groups, each containing 18 ejaculates, were studied in both good and poor freezable semen. The cysteine hydrochloride was added (5 mM) to the conventional Tris extender, fast freezing and followed by thawing and post-thawed seminal parameters were measured. Out these parameters the sperm viability, hypo-osmotic swelling, positive sperm, Vanguard distance travelled by sperm (mm h^{-1}) in cervical mucus and nuclear fragmentation showed significant (p<0.05) difference between the two groups in both freezable bull semen, whereas the other parameters such as total motility and mitochondrial membrane potential were non-significantly higher and total sperm abnormality, loss of acrosomal integrity, malondialdehyde production were non-significantly lower in cysteine hydrochloride treated semen in both freezer. The addition of cysteine hydrochloride had maintained the functional parameters of good and enhances that in the poor freezable semen. Thus the cysteine hydrochloride improves post-thawed sperm functional parameters in poor freezable semen, suggesting that addition of cysteine hydrochloride in semen extender may improve fertility in these bulls.

1. Introduction

The conception rate in frozen thawed semen has been reported to be low (Waberski, 2007). The process of cryopreservation exerts physiological, osmotic and chemical stress on the sperm membrane and sperm structure, which may result into damage of spermatozoa and post-thawed quality of spermatozoa (Ozkavukcu et al., 2008). The good semen has the ability to tolerate various adverse effects during freezing process like osmotic stress, solution effect and large ice crystal formation (Morris et al., 2007; Ozkavukcu et al., 2008). The spermatozoa membrane has highly unsaturated fatty acid content (Sinha et al., 1996), and lipid peroxidation of spermatozoa membrane eventually leads to apoptotic cell changes and loss of sperm function (Sikka, 1996) in post-thawed semen samples. Usually, the semen additives may maintain frozen-thawed seminal quality and improve the conception rate (Perumal et al., 2011ab). The cryopreservation reduces the content of thiols such as cysteine hydrochloride and reduced glutathione of spermatozoa and seminal plasma (Bilodeau et al., 2001; Gadea et al., 2008) and leading to changes in membrane transportation (Alvarez et al., 1982), affecting fertility of the spermatozoa. Both cysteine hydrochloride and N-acetyl-cysteine are precursors of intra-cellular glutathione biosynthesis (Meister et al., 1983). Perusal of literature revealed paucity of information on the ability of cysteine hydrochloride in improving freezability of poor freezable bull by maintaining the sperm membrane integrity during cryopreservation and thawing. Hence, the objective of this study was to assess the role of cysteine hydrochloride in improving semen quality in poor freezable Jersey crossbred bull semen.

2. Materials and Methods

The experiment was conducted in 3 good freezable and 3 poor freezable Jersey crossbred bulls belonging to Frozen Semen Bank, Cuttack under Animal Husbandry and Veterinary Services, Government of Odisha, India during November, 2007 to July, 2008. The experimental bulls of 4-6 years of age with good body condition (score 5-6) were selected and maintained under optimum management practices as per the standard criteria fixed for maintenance of breeding bulls in bull stations.

The pre-freezing seminal parameters of both groups of semen had >60% progressive forward motility, less than 20% total abnormality. The good and poor freezable bull semen was classified based on post-thawed motility parameter. Those bulls provided consistently more than 40% post-thawed motility were considered as good freezer bulls (n=3), whereas those bulls with less than 40% post-thawed motility were considered as poor freezer bulls (n=3). Ejaculates (6) from each bull were collected twice in a week. Each ejaculate was split in to 2 equal parts to use 1 part as control without cysteine hydrochloride and another part with cysteine hydrochloride @ 5mM for cryopreservation and subsequent evaluation. All experimental semen samples were extended with Tris extender and frozen by rapid freezing method in mini straws and cryopreserved using programable freezer and stored in liquid nitrogen. Then frozen-thawed seminal characters like individual motility, percentage of live sperm, abnormal spermatozoa and acrosomal integrity were studied by staining with eosin- nigrosin and giemsa staining methods, respectively. The *in vitro* fertility test like Vanguard distance travelled by sperm in cervical mucus penetration test, percentage of hypo-osmotic swelling test (HOST), positive sperm (at 150 mOsm) were assessed as per Jeyendran et al. (1984). The lipid peroxide assay (MDA) was conducted as per Suleiman et al. (1996). Mitochondrial membrane potential was assessed by using JC-1 and nuclear integrity by Fuelgen's Staining Technique (Selvaraju et al., 2008). The results were analyzed statistically after arcsine transformation of percentage data using SPSS 15.0 software.

3. Results and Discussion

The post-thawed seminal parameters such as sperm viability, hypo-osmotic swelling, positive sperm, Vanguard distance travelled by sperm (mm h^{-1}) in cervical mucus and nuclear fragmentation has shown significant ($p<0.05$) difference between the two groups in both freezable bull semen, whereas the other parameters such as total motility and mitochondrial membrane potential were non-significantly higher and total sperm abnormality, loss of acrosomal integrity, malondialdehyde production were non-significantly lower in cysteine hydrochloride treated semen in both freezer (Figure 1 & 2). The results of the study were in agreement with the findings of Uysal and Bucak (2007) and Bucak et al. (2008) for ram; Trimeche et al. (1999) and Khlifaouia et al. (2005) for stallion and Kundu et al. (2001) for goat sperm. The cysteine hydrochloride treated group had non-significantly higher post-thawed motility as compared to control group in good and poor freezable bull semen, which is very comparable to the earlier findings (Bilodeau et al., 2001; Jain and Arora, 1998; Uysal et al., 2007). The cysteine hydrochloride treated sperm has more and fast forward movement as compared to control group because this amino acid protects calcium-dependent ATPase of sarcoplasmic reticulum (Lalonde et al., 1991) and certain enzymes (Noguchi et al., 1971) during the changes of state of the freezing medium. Cysteine is a low molecular weight amino acid containing thiols; it is a precursor of intra-cellular glutathione biosynthesis, and increases the GSH level, which neutralizes and prevents the formation of free radical and maintains the membrane integrity and increases the efficiency of flagellar motion efficiently in fluid medium. Moreover, cysteine hydrochloride also improves the efficiency of mitochondria (Perumal et al., 2011a, b) (Figure 3) and also protects the other parts of sperm membrane to maintain the motility by enhanced production of glutathione and it acts as antioxidants to reduce the free radical formation in semen and sperm (Sheshtawy et al., 2008). During freezing, the spermatozoa membrane is damaged resulting in leakage of enzymes. The present study revealed that addition of cysteine hydrochloride in freezing extender prevents leakage of enzymes through the action of membrane stabilizer as stated by Matilde et al. (2005). The total live sperm percentage of cysteine hydrochloride treated group was higher than control group of different freezable bulls (Funahashi et al., 2005; Szczesniak-Fabianczyk et al., 2006). Addition of cysteine hydrochloride proportionally enhanced the viability in good freezable semen and maintained the viability in poor freezable bull sperm. The present observation in the loss of acrosomal integrity in post-thawed semen was partially in accordance with the findings of Uysal et al. (2007). The frozen thawed value of HOST revealed a significant difference ($p<0.05$) between experimental groups in good freezable semen. These observations indicated that the cysteine hydrochloride acts as membrane stabilizer (Matilde Maiorino et al., 2005; Funahashi et al., 2005; Szczesniak-Fabianczyk et al., 2006). The reduced lipid peroxidation potential of the cysteine hydrochloride treated semen samples suggested that cysteine hydrochloride might protect the spermatozoa from membrane damage by inhibiting the lipid peroxidation process as suggested by Mazur et al. (2000). The protective effects of cysteine hydrochloride is due to their ability to form a layer on the spermatozoa surface, as these positively charged molecules can combine with the phosphate groups of sperm plasma membrane phospholipids (Kundu et al., 2001; Anchordoguy et al., 1988). The present study revealed that bull semen extended with cysteine hydrochloride has showed higher percentage of cervical mucus penetration than control group semen. The migration of the Vanguard distance was higher due to enhanced straight forward motility, which was ascribed to normally produce adequate mucus penetration (Munsi et al., 2007). The cysteine hydrochloride incorporation to the extended semen might have potentiated the viability of the sperm with respect to its motility and morphological

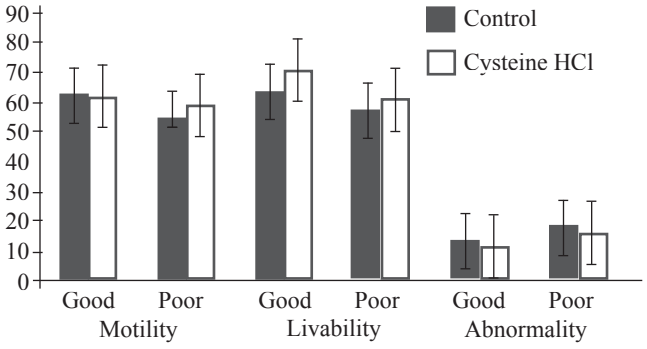

Figure 1: Post-thawed motility, livability and abnormality of cysteine hydrochloride treated semen (p<0.05)

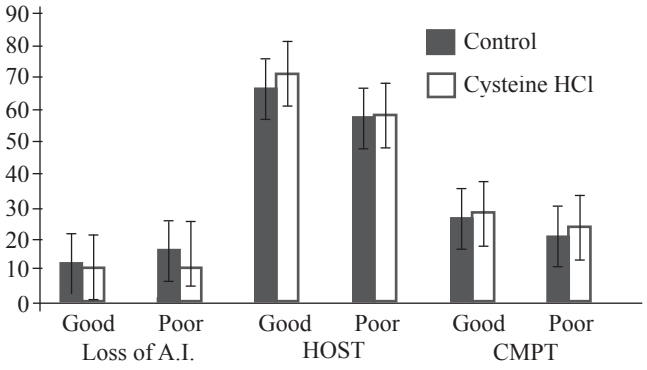

Figure 2: : Post-thawed loss of acrosomal integrity, hypo-osmotic swelling test and cervical mucus penetration test of cysteine hydrochloride treated semen (p<0.05)

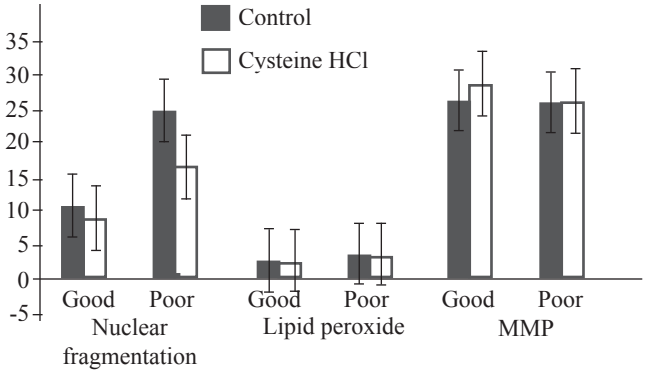

Figure 3: Post-thawed nuclear fragmentation, malondialdehyde production and mitochondrial membrane potential (MMP) of cysteine hydrochloride treated semen (p<0.05)

integrity (Funahashi et al., 2005), which was reflected by the highest rate of penetration through the cervical mucus in the present course of investigation.

4. Conclusion

The study concludes that the addition of cysteine hydrochloride in semen extender had maintained the functional parameters of good, but enhanced the poor freezable semen and might improve fertility in these bulls.

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