International Journal of Science, Environment and Technology, Vol. 7, No 2, 2018, 554 – 560
ISSN 2278-3687 (O)
2277-663X (P)

SEMEN ADDITIVES FOR IMPROVES MOTILITY AND FERTILITY OF BOVINE SPERMATOZOA- A REVIEW

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Abstract: Bovine species are considered as back bone of agriculture in India, population dynamics of bovine species now a day's decreased due to failure of conception. Failure of conception in female animals revealed poor quality semen as the cause of failure of conception, it suggested that spermatozoa of the mammalian species are highly sensitive to oxidative damage due to presence of high lipid bi-layer of plasma membrane. Even though the semen possessing some endogenous anti-oxidant, sperm highly prone to oxidative damage during storage of semen. Semen additives are the substance added to the semen during processing of semen for preservation at different temperature to prevent the oxidative damage in turn improve the keeping quality of semen for longer period of time without any deleterious effects. Semen additives are not only scavenging the free radicals but also improve the sperm motility and fertility. In the present paper reviewed the various semen additives that were commonly used in semen preservation process. In this present review gives overall idea about oxidative status of sperm and different classes of semen preservative that were used in semen preservation process.
Keywords: Antioxidant; Oxidative damage; Semen additives; Sperm motility.

INTRODUCTION

Artificial insemination(AI) is one the important assisted reproductive technologies, which causes widespread propagation of semen, limiting the spread of venereally transmitted diseases and mainly facilitating improvement on genetic makeup of animal. Many research workers documented buffalo semen have poor keeping quality of and freezability (Sansone *et al*., 2000; Chaudhari *et al*., 2015). It suggested that spermatozoa of the mammalian species are highly sensitive to oxidative damage due to presence of high lipid bi-layer of plasma membrane. Naturally bovine semen have defence system against oxidative damage, it is not tolerate under refrigeration and cryopreservation temperature. The extender/diluents used in preservation of semen is considered as important factor, which should has ambient P^H and

*Received Feb 18, 2018 * Published April 2, 2018 * www.ijset.net*

buffering capacity followed by appropriate osmolality to protect spermatozoa from the cryogenic injury. Oxidative damage of bull and buffalo spermatozoa during preservation could be hindered by addition of suitable additives to the semen diluents/extenders (Ansari *et al*., 2011).

Oxidative damage result in a decrease in intracellular ATP levels which in turn decreases sperm motility and also induces lipid peroxidation in polyunsaturated fatty acids (PUFA) rich plasma membrane of spermatozoa. Such events have been related with increased permeability of plasma membrane, enzyme deactivation and production of metabolic end product that highly toxic to spermatozoa (Spermicidal).

Verma and Kanwar, (1998) opined that lipid peroxidation leads to abnormal acrosomal reaction and excessive loss of plasma membrane fluidity culminating in a loss of freezing capability of spermatozoa. Complete deactivation of oxidative stress is not possible; but certain measures are followed to prevent the oxidative stress to the sperm as well as improve the motility and fertility of spermatozoa by adding various motility enhancing factors like anti-oxidants, antioxidant preservatives and methylxanthines.

DIFFERENT CLASSES OF SEMEN ADDITIVES

Different classes of semen additives were used for prevention of oxidative damage caused during different method of preservation of semen. They were categorised into,

1.Antioxidant- Vitamin-E, Ascorbic acid.

2.Antioxidant Preservatives- Butylated hydroxy toluene (BHT), Butylated hydroxy anisole

3.Methylxanthine- Pentoxifylline (PTX) and Caffeine.

4.Trace Elements- Copper, Zinc, Selenium.

5.Enzymes/ Co-factor- Superoxide desmutase (SOD) and Glutathione peroxidase (GPX)

6.Amino acids/Proteins- Cysteine, Taurine, Hypotaurine, Bovine serum albumin, Platelet activating factors.

7.Sugar/Polysaccharides- Trehalose, Hyaluronic acid.

Butylated Hydroxy Toluene (BHT)

Butylated hydroxy toluene (BHT) act as a anti-oxidant preservative, antiviral and antimicrobial agent. Long term storage of unsaturated fatty acid results in rancidity, that can be prevented by addition of BHT. BHT is an organic soluble molecules, used to stop the auto-oxidation of lipid bi-layer and membrane of sperm cells (Hammerstedt *et al*., 1978). BHT has ability to readily incorporates into sperm membranes and prevent the sperm from cold shock after exposed to cold storage (Anderson *et al*., 1994).

Semen diluents and spermatozoa highly affected by reactive oxygen species (ROS), that can be minimized by addition of antioxidant preservatives like BHT, which role considered to be scavenging of free radicals (Killian *et al*., 1989). Anderson *et al*., (1994) opined that BHT improved the sperm viability during thawing and freezing process through increase the fluidity and render the sperm to less susceptible to cold shock. Utmost BHT act as a antiviral agent, so reduce the risk of transmitting viral diseases to cow during AI.

Addition of egg yolk in BHT treated spermatozoa improved the motility by synergistic action of both lipid vesicle in milk and egg yolk interact with BHT to guard the spermatozoa from cold induced shock (Graham and Hammersted, 1992). Bull spermatozoa treated with BHT of 0.5 and 0.75 mM/L had no effect on motility in that ration (Killian *et al*., 1989). Recent document revealed that 1.5mM BHT/L enhances the motility, acrosome integrity followed by plasma membrane integrity and liveability of spermatozoa at refrigerator temperature (Pankaj, 2006; Bhakat *et al*., 2011).

Methylxanthines:

Among various methylxanthines, Pentoxifyyline (PTX) has been used as additive to the semen. Aitken *et al*., (1993) documented that stimulating effect of pentoxifyine on capacitating process and acrosome reaction, thereby increasing the sperm fertilizing ability (Esteves *et al*., 2007).

PTX is a methylxanthine phosphodiesterase enzyme inhibitors which decreases the level of superoxide anions responsible for DNA apoptosis when used at a minimum concentration of 3.6mM PTX/L (Maxwell *et al*., 2002). It inhibit the phosphodiesterase enzyme resulted in accumulation of intracellular calcium(Ca^{2+}) ions followed by intracellular adenosine triphosphate (ATP) level will be increases that lead to enhancement of sperm motility (Sikk and Hellstrom, 1991). Addition of PTX increases the post thaw motility of spermatozoa in human (Aribarg *et al*., 1994) and in cat semen (James *et al*., 1994). Reduced or decreased motility of spermatozoa as called as *asthenozoospermia,* that can be improved by addition of PTX (Yunes et al., 2005). Recent document revealed that 3.6mM PTX/L enhances the motility, acrosome reaction followed by plasma membrane integrity and liveability of spermatozoa at refrigerator temperature (Bhakat *et al*., 2011).

Platelet Activating Factors (PAF_S):

Platelet activating factors has pleiotropic biological properties that signalling phospholipids in addition to thrombocyte (platelets) activation (Roundebush, 2007). Actually PAF is produced naturally by sperm itself, but that is not sufficient for effective function of

spermatozoa in preservation techniques. Roundebush and Purnell, (2000) opined that PAF has influences the ovulation, fertilization, implantation and parturition in female where as in male it significantly improve the sperm motility. Moreover additionally endogenous platelet activating factor act as a biomarker for normal function of spermatozoa (Levine *et al*., 2002)

α-Tocopherol (Vit-E):

Vitamin-E is important body cellular constituents for protection against oxidative stress in both feeds and biological systems. α-tocopherol act as a liphophillic compound result in scavenging of reactive oxygen species (ROS) and interrupt the propagation of lipid peroxidation process. Vitamin-E protects the sperm plasma membrane through inhibition of lipid peroxidation (Pena *et al*., 2003) resulted in increased intracellular ATP level, decreased abnormal acrosome reaction and spermatozoa motility increased (Breininger *et al*., 2005).

Recent documents suggested a positive response on maintenance of sperm motility in liquid semen storage when addition of Vit-E during storage of ram, turkey and boar semen (Sarlos *et al*., 2002 ; Douard *et al*., 2004; Breininger *et al*., 2005). Recent document revealed that 1mgVit-E/ml enhances the motility, acrosome integrity followed by plasma membrane integrity and liveability of spermatozoa at refrigerator temperature (Bhakat *et al*., 2011).

Zinc Sulphate ($ZnSo_4$):

Zinc is considered as the important trace elements in the biological system when compared to the other trace elements. Because zinc deficiency causes infertility in most animals. Utmost zinc act as a co-factor in spermatogenesis and need for increasing the concentration of spermatozoa (Wong *et al*., 2001). Zinc also necessary for maintain the stability of sperm chromatin and repair of DNA damage (Barratt *et al*., 2010), moreover zinc posses anti-oxidant capacity (Dissanayake *et al*., 2006). Dorostakar *et al*., (2014) evaluated that adding 0.288mg of Zn /L to the extender gives a better sperm preservation in the freezing process.

Other anti-oxidant and Amino acids:

Glutathione, oxidized glutathione, Cysteine, Taurine, Hypotaurine, Bovine serum albumin (BSA), trehalose, hyaluron were tested to minimize the oxidative damage during cooling, freezing and thawing on bull semen (Uysal *et al*., 2007), water buffalo semen (Ghosh and Data, 2003), stallion semen (Ball et al., 2001), goat semen (Salvador *et al*., 2006) and ram semen (Paulenzen *et al*., 2002).

CONCLUSION

Among the various semen additives Butylated hydroxy toluene (BHT) and Vitamin-E considered to be the valuable semen additives for improve the live sperm concentration, decline the abnormal acrosome reaction and reduces the sperm abnormality followed by

improve the motility and fertility characteristics of spermatozoa. It was mostly used by recent researchers for improves the semen quality.

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