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Research Article

EFFECT OF ADDING TAURINE AND TREHALOSE ADDITIVES ON OXIDATIVE STRESS OF SPERMATOZOA OF KANKREJ BULLSEMEN DURING CRYOPRESERVATION

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Abstract- Total 24 ejaculates, 8 each from 3 mature Kankrej bulls were divided into 3 equal aliquots, the first aliquot was added with TFYG diluent with Taurine (50mM) second with Trehalose (100mM) as semen additives and third serve as a control (without additive) and each aliquot was evaluated at post diluted, post equilibrated and post thawed stages for oxidative parameter *viz*. Lipid peroxidation (LPO) and Glutathione reductase (GSH). The level of LPO was significantly lower (P <0.05) and GSH were significantly higher (P < 0.05) in presence of Taurine as compared to Trehaloseand control at post thaw stage of semen cryopreservation. It can be concluded that Taurine seems to control the oxidative stress more efficiently as indicated by significant increase in glutathione reductase and decline in malondialdehyde level at post thawed stage of cryopreservation in Kankrej bull semen.

Keywords- Additives, Oxidative stress, Kankrej bull, Cryopreservation.

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Introduction

Kankrej is an important dual-purpose cattle breed of India. Kankrej is very powerful draft cattle of Gujarat and have fair milk production. They have been well adopted in North Gujarat and have proved to be superior to crossbreds in respect to milk production and disease resistance. The efficacy of semen extender can be further amplified by adding antioxidants such as Taurine and Trehalose [1]. Presence of high phospholipids and unsaturated fatty acid contents in mammalian spermatozoa makes them more responsive to lipid peroxidation (LPO) [2]. For long term semen storage, these antioxidants levels are inadequate and may excess oxidative stress during the cryopreservation. It has also been reported that freeze-thaw process may also elevate reactive oxygen species (ROS) in semen and leads to further damage [3]. Antioxidants provide resistance to spermatozoa against oxidative stress [4] and prevent the excessive free radical's production [5]. Trehalose, a non-reducing disaccharide, which presumably involves a stabilization of certain cell proteins and/or lipid in membranes during stresses such as cryopreservation, heat, desiccation or oxidative stress [6]. When Trehalose was added in hypertonic solutions, it showed a synergic effect with glycerol which is used as a cryoprotectant in order to avoid intracellular ice crystal formation [7].

Taurine is an intracellular amino acid found in majority of the mammalian tissues and plays a role in cell proliferation, viability, osmoregulation and prevents injuries induced by oxidants in tissue [8]. Taurine also maintains the stability of bio membranes, scavenges ROS, minimizes the end products of lipid peroxidation [9], modulates Ca2+ uptake [10] and inhibits protein phosphorylation [11].

The addition of antioxidants such as Taurine and Trehalose to bovine sperm [12] has been shown to protect sperm against the harmful effects of ROS and improve sperm motility and membrane integrity during sperm storage. There for important goal for the present study was to determine the cryoprotective and antioxidant activity of Taurine and Trehalose on frozen thawed Kankrej bull semen.

Material and Methods

Total three Kankrej bulls, ranging from 6 to 8 years of age and clinically normal, were selected as semen donors from livestock research station, S. K. NAGAR, S.D.A.U. All the standard procedures from semen collection to its storage in nitrogen were followed in strict aseptic condition. A total of eight ejaculates were obtained from each bull for eight weeks. Each ejaculation sample was divided in three equal aliquots. Aliquot-1 was diluted with Taurine (50 mM) added TFYG diluent, aliquot-2 was diluted with TFYG diluent added with Trehalose (100 mM) and aliquot-3 was diluted with TFYG diluent without any additive and served as control. The dilution rate was calculated keeping in view the sperm concentration per dose of diluted semen. All the aliquots of known volume and concentration were centrifuged at 3000 r.p.m. for 10 minutes to separate out the seminal plasma. The plasma was separated at post-dilution, post-equilibration and post-thawing stages of cryopreservation for the study of oxidative stress parameter. The data obtained for oxidative stress parameter were analyzed statistically and expressed as Mean ± S.E. for post- dilution, post-equilibrated and post-thaw semen. Three factorial CRD (Completely Randomized Design) followed by Duncan New Multiple Range Test (DNMRT) used for post-diluted, post-equilibrated and post-thawed semen to determine the difference between different groups and stages using the methodology as described by Snedecor and Cochran [13].

Results and Discussion

The overall mean lipid peroxidation (LPO) levels in Taurine group was significantly (P<0.05) lower at post-dilution, post-equilibration and post-thaw stages of cryopreservation as compared to that of the Trehalose and control groups [Table-1].

The overall mean glutathione reductase (GSH) value in Taurine group was significantly (P<0.05) higher at post-equilibration and post-thaw stages of

cryopreservation as compared to that of the Trehalose group whereas, it was significantly (P<0.05) higher at all the stages of cryopreservation as compared to that of the control groups [Table-1].

Table-1 *Lipid peroxidation (µmol/ml) and Glutathione reductase (U/L) in different groups at various stages of cryopreservation in Kankrej bull semen.*

Semen diluent additives	Lipid peroxidation (µmol/ml)			Glutathione reductase(U/L)		
	PDS	PES	PTS	PDS	PES	PTS
Taurine50mM (n=24)	41.94 ± 0.46^{a}	33.13 ± 0.54^{a}	16.70 ± 0.38^{a}	56.30 ± 0.49^{a}	65.97 ± 0.40^{a}	85.34 ± 0.62^{a}
Trehalose100mM (n=24)	43.50 ± 0.51^{b}	34.69 ± 0.50^{b}	20.63 ± 0.44^{b}	55.20 ± 0.38^{a}	64.31 ± 0.43^{b}	83.87 ± 0.61^{b}
Control	47.74 ± 0.62^{c}	37.18 ± 0.53^{c}	24.40 ± 0.47^{c}	40.10 ± 0.43^{b}	49.46 ± 0.39^{c}	61.91 ± 0.48^{c}

Note: Means with different superscripts within row differ significantly at 5% level.

Level of the malondialdehyde in Taurine group was significantly lower($P < 0.05$) than the Trehalose group in the present study at all stages during preservation or cryopreservation the semen is unguarded to cold shock at atmospheric oxygen which in turn increases the receptivity to lipid peroxidation due to higher production of reactive oxygen species [14]. Therefore, in the present study addition of Taurine and Trehalose in semen might be a beneficial factor in avoiding the process of damage and reduce generation of ROS which would otherwise have negatively affected the spermatozoa [15, 16].

The GSH sustained the ability in the maintenance of sperm membrane integrity and individual motility. So, higher the GSH values in the semen might be a factor in making the membrane of sperm more resistant to the spontaneous lipid peroxidation that destroys the structure of the lipid matrix and is associated with the loss of motility [15]. Trapping of the free radicals by Taurine and Trehalose, thereby alleviating GSH consumption by the enzymatic antioxidant defences might be implicated in higher GSH levels observed in the present study.

However, Taurine seems to control the oxidative stress more efficiently as indicated by significant increase in glutathione reductase and significant decline in malondialdehyde level at post thawed stage of cryopreservation in Kankrej bull semen.

Conclusions

Taurine controls the oxidative stress as indicated by an increase in GSH and a decline in MDA level at post thawed stage of cryopreservation in Kankrej bull semen.

Taurine as an additive has a better antioxidant property in comparison with Trehalose in TFYG extender for cryopreservation of Kankrej bull semen

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Abbreviations:

TFYG- Tris Fructose Egg Yolk Glycerol
LPO - Lipid Peroxidation
GSH – Glutathion Reductase
mM - milli Mole
PTS- Post Thaw Stage
µmol/ml- Micro mole per milliliter
U/L - Unit per Liter
PDS- Post Dilution Stage
PES- Post Equilibration Stage

Conflict of Interest: None declared

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