

Original Research Article

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Effect of Different Concentrations of Taurine on Certain Biochemical Parameters During Cryopreservation of Gir Bull Semen

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ABSTRACT

A Total 8 semen sample from four healthy Gir breeding bulls were collected at weekly interval for two weeks period using of artificial vagina method for evaluation of semen biochemical attributes at various stages of cryopreservation of Gir bull semen. The overall mean values for oxidative stress parameters viz. lipid peroxidation and glutathione reductase were $51.14 \pm 0.18 \mu\text{mol/ml}$ and $32.24 \pm 0.64 \text{ U/L}$, respectively in neat semen samples. The difference was non-significant among the bulls for the glutathione reductase, while significant ($P < 0.05$) difference was found for the lipid peroxidation. Mean Malondialdehyde (MDA) level were $30.74 \pm 0.55 \mu\text{mol/ml}$ in 50mM Taurine group at post-thaw stages of cryopreservation, and were significantly ($P < 0.05$) lower as compared to that of the other groups. Mean glutathione reductase levels in 50 mM Taurine group were $64.76 \pm 0.78 \text{ U/L}$ at pre-freeze stages of cryopreservation, and were significantly ($P < 0.05$) higher as compared to that of the other groups. Mean Malondialdehyde (MDA) level was $15.95 \pm 0.74 \mu\text{mol/ml}$ in 50 mM Taurine group at post-thaw stages of cryopreservation, and were significantly ($P < 0.05$) lower as compared to that of the other groups. Mean glutathione reductase levels in 50 mM Taurine group were $80.25 \pm 4.85 \text{ U/L}$ at post-thaw stages of cryopreservation, and were significantly ($P < 0.05$) higher as compared to that of the other groups.

Keywords

Gir bull, Semen, taurine, Glutathione reductase, Lipid peroxidation

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Introduction

Gir is well known milch breed of Indian. It well known for its milk production, docility and emotional temperament. This breed is found in and around districts like Junagadh, Amreli, Bhavnagar, Gir Somnath, Rajkot, Porbandar and some parts of Jamnagar, Morabi, Surendranagar district of Saurashtra. This breed is also locally known as Bhodali,

Kathiyawari, and Sorathi. Pure Gir heard is well maintained at Cattle Breeding Farm Junagadh, JAU.

One of the most important factors contributing to poor quality semen has been reported to be oxidative stress (Bucak *et al.*, 2010). Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen derived oxidants

commonly known as ROS (Sikka *et al.*, 1995). Gametes are susceptible to reactive oxygen species (ROS) attack. When manipulated *in vitro* during assisted reproductive techniques, these cells run the risk of generating and being exposed to supra-physiological level of ROS (Du Plessis *et al.*, 2008). Among various causes, oxidative stress (OS) has been attributed to affect the fertility status and physiology of spermatozoa (Agarwal *et al.*, 2008).

Moreover, bovine spermatozoa are prone to membrane damage due to high content of polyunsaturated fatty acids in the sperm membrane and lack of a significant antioxidant system in the cytoplasm (Bailey *et al.*, 2000). This makes bovine spermatozoa particularly susceptible to Lipid Peroxidation (LPO) in presence of ROS (Sinha *et al.*, 1996).

Bansal and Bilaspuri, (2011) reviewed the impact of oxidative stress and antioxidants on sperm function and explained effects of various types of antioxidants. The effects of LPO include irreversible loss in motility, damage to the sperm DNA, and decreased fertility (Perumal *et al.*, 2011 and Maxwell and Watson, 1996). In recent years, studies have also been conducted on bovine semen diluents, including additives such as, taurine, trehalose, selenium, GSH, GPX, CAT, SOD, and surfactant compounds, so as to improve motility, viability, and membrane integrity of spermatozoa (Aisen *et al.*, 2002 and Uysal *et al.*, 2005).

The addition of antioxidants such as taurine to ovine sperm (Bucak and Tekin, 2007), feline sperm (Baran *et al.*, 2009), and rabbit sperm (Alvarez *et al.*, 1987) has been shown to protect sperm against the harmful effects of ROS and improve sperm motility and membrane integrity during sperm liquid storage.

Keeping in mind the above factors a study was planned to study the effect of different concentrations of taurine as an additive in and romed extender during the cryopreservation of Gir bull semen

Materials and Methods

A total of four Gir bulls aged between 4 to 6 years of Cattle Breeding Farm, Junagadh were used for the study. All bulls included in study had sound general reproductive health and were maintained under uniform managerial conditions

The semen was collected at homosexual mount by Artificial Vagina (Danish model) once a week. A total of 8 ejaculates, 4 from each bull were collected for assessment and preservation. Immediately after collection, semen collection tubes were placed in water bath at 35°C. The collected semen was examined microscopically and all routine seminal traits were recorded. After initial dilution and evaluation of semen ejaculates with more than 70 per cent initial progressive motility, were divided in four equal aliquots. Subsequently, Aliquot-1 was diluted with 25 mM Taurine (25 mM Taurine group), Aliquot-2 was diluted with 50 mM Taurine (50 mM Taurine group), Aliquot-3 was diluted with 75 mM Taurine (75 mM Taurine group) and Aliquot-4 was kept without any additive which was served as control. The dilution rate was calculated automatically by the photometer (Accucel, IMV, France) against 530nm wavelength. After dilution, each semen aliquots were filled and sealed in French Medium transparent Straw (0.5 ml capacity, TBS™, IMV, France) by Automatic Filling and Sealing Machine (MRS1 Dual, IMV, France) and printed using straw printer (IMV, France). Straws were then arranged in Freezing Rack and were transferred to Cold Handling Cabinet (Macro scientific Pvt. Ltd, New Delhi.) already maintained at 4°C kept

for 4 hours of equilibration period. Then after straws were vapour frozen using conventional method and then all the straws were submerged in Liquid Nitrogen at -196°C for storage. After cryopreservation, straws were thawed at 37°C for 30 seconds in a water bath to evaluate post-thaw individual motility, sperm viability, sperm abnormality, plasma membrane integrity (HOST) and acrosomal integrity. The seminal plasma was collected from neat semen and cryopreserved semen straws at pre-freeze and post-thaw stages by centrifuging at 3000 rpm for 10 minutes and evaluated for biochemical attribute like MDA and GR by using standard kit provided by sigma Aldrich (Saint Louis, USA)

Results and Discussion

Biochemical parameter values for neat Gir bull semen

Lipid peroxidation (LPO)

The overall mean Malondialdehyde (MDA) values of semen samples was 51.14 ± 0.18 $\mu\text{mol/ml}$ with a range of 50 to 52 $\mu\text{mol/ml}$. The mean values of Malondialdehyde (MDA) were 50.5 ± 0.03 , 51.4 ± 0.02 , 51.51 ± 0.13 and 50.3 ± 0.01 for the Pankaj, Pratap, Sarang and Vasu bulls, respectively. The mean Malondialdehyde (MDA) differed significantly among the bulls (Table 1). Similar findings were reported by Shaikh (2014) in Kankeraj breeds of cattle.

Glutathione reductase (GR)

The overall mean glutathione reductase (GR) of semen was 32.24 ± 0.64 U/L with a range of 31.16 to 36.12 U/L. The mean values of glutathione reductase were 33.51 ± 2.91 , 31.50 ± 0.25 , 32.62 ± 0.02 and 31.31 ± 0.015 U/L for the Pankaj, Pratap, Sarang and Vasu bulls, respectively (Table 1). Value observed for Glutathione reductase in the present study is

in agreement with the reports of the Shaikh (2014) in Kankeraj bulls who has reported the GR level as 30.14 ± 0.06 U/L.

Effect of taurine as a semen additive during cryopreservation on biochemical parameter of Gir bull semen

Lipid peroxidation (LPO)

The mean Malondialdehyde (MDA) values in pre freeze stage and post thaw stage were 36.33 ± 0.76 and 24.53 ± 0.63 $\mu\text{mol/ml}$ in 25mM Taurine group; 30.74 ± 0.55 and 15.95 ± 0.74 $\mu\text{mol/ml}$ in 50mM Taurine group; 37.6 ± 0.63 , and 28.48 ± 0.55 $\mu\text{mol/ml}$ in 75 mM Taurine group and 38.57 ± 0.70 and 28.07 ± 1.26 $\mu\text{mol/ml}$ in control group at pre-freeze and post-thaw stages of cryopreservation, respectively. The mean Malondialdehyde (MDA) value in 50 mM Taurine group was significantly ($P < 0.05$) lower at pre-freeze and post-thaw stages of cryopreservation as compared to that of the 25mM Taurine, 75 mM Taurine and control groups. It was also observed that Malondialdehyde (MDA) values was found to be significantly ($P < 0.05$) higher in all groups at pre-freeze stage as compared to post-thaw stage of cryopreservation in the present study (Table 2).

These findings are in accordance with (Chhillar *et al.*, 2012) who also have reported that the addition of 50mM Taurine to the freezing extender resulted in significantly decreased Malondialdehyde (MDA) at post-thaw stage in Karan-Fries bulls.

Similarly, Serpil *et al.*, (2009) have also reported a significantly lower Malondialdehyde (MDA) at post-thaw stage but at lower concentration of Taurine in cattle bull. Whereas, Atessahin *et al.*, (2008) have reported a significantly lower Malondialdehyde (MDA) at post-thaw stage but using higher concentration (75mM) of

Taurine in Angora Goat. Perumal *et al.*, (2013) have also found significantly lowered Malondialdehyde (MDA) level at 50 mM

Taurine concentration but for the liquid storage of mithun bull semen.

Table.1 Biochemical parameters of neat semen of Gir bulls (Mean ± SE)

Bull Name (n=4)	Oxidative stress parameters	
	Lipid Peroxidation (µmol/ml)	Glutathione Reductase (U/L)
Pankaj (n=2)	50.50 ± 0.03 ^a	33.51 ± 2.91 ^a
Pratap (n=2)	51.40 ± 0.02 ^{ab}	31.50 ± 0.25 ^a
Sarang (n=2)	51.51 ± 0.13 ^b	32.62 ± 0.02 ^a
Vasu (n=2)	50.30 ± 0.01 ^{ab}	31.31 ± 0.01 ^a
Overall (n=8)	51.14 ± 0.18	32.24 ± 0.64

Means with different superscripts within column and mean with different subscripts within row differ significantly at (P < 0.05) level

Table.2 Lipid peroxidation (µmol/ml) in different groups at various stages of cryopreservation in Gir bulls semen (Mean ± SE)

Groups (n=8)	Stages of cryopreservation		
	Pre- freeze Stage (PFS) at 4 ⁰ C	Post-Thaw Stage (PTS) at 37 ⁰ C	Overall Mean
Taurine 25 mM	36.33± 0.76 ^{A_a}	24.53 ± 0.63 ^{A_b}	30.43 ± 1.59
Taurine 50 mM	30.74 ± 0.55 ^{B_a}	15.95 ± 0.74 ^{B_b}	22.10 ± 2.06
Taurine 75 mM	37.60 ± 0.63 ^{A_a}	28.48 ± 0.55 ^{C_b}	33.08 ± 1.25
Control	38.57 ± 0.70 ^{A_a}	28.07 ± 1.26 ^{C_b}	33.32 ± 1.52

Means with different superscripts within column and Means with different subscripts within raw differ significantly (P<0.05).

Table.3 Glutathione reductase (U/L) in different groups at various stages of cryopreservation in Gir bulls semen (Mean ± SE)

Groups (n=8)	Stages of cryopreservation		
	Pre- freeze Stage (PFS) at 4 ⁰ C	Post-Thaw Stage (PTS) at 37 ⁰ C	Overall Mean
Taurine 25 mM	49.77 ± 0.72 ^{A_a}	62.91 ± 0.73 ^{A_b}	56.34 ± 1.76
Taurine 50 mM	64.76 ± 0.78 ^{B_a}	80.25 ± 0.85 ^{B_b}	72.50 ± 3.09
Taurine 75 mM	48.39 ± 0.78 ^{A_a}	59.94 ± 0.91 ^{A_b}	54.17 ± 1.60
Control	47.29 ± 0.56 ^{A_a}	59.20 ± 0.76 ^{A_b}	53.24 ± 1.60

Means with different superscripts within column and Means with different subscripts within raw differ significantly (P<0.05)

During preservation or cryopreservation, the semen is exposed to cold shock at atmospheric oxygen which in turn increases the susceptibility to lipid peroxidation due to higher production of reactive oxygen species (Perumal *et al.*, 2009).

The free radicals are known to be involved in lipid peroxidation as well as DNA and sperm membrane damages which may lead to decreased sperm motility or cell death. Therefore, in the present study addition of Taurine 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 (Chiller *et al.*, 2012).

Glutathione reductase (GR)

The mean glutathione reductase (GR) values in for Pre freeze stage and post thaw stage were 49.77 ± 0.72 and 62.91 ± 0.73 U/L in 25 mM Taurine group; 64.76 ± 0.78 and 80.25 ± 0.85 U/L in 50 mM Taurine group; 48.39 ± 0.78 and 59.94 ± 0.91 U/L in 75 mM Taurine group and 47.29 ± 0.56 and 59.20 ± 0.76 U/L in control group at pre-freeze and post-thaw stages of cryopreservation, respectively.

The mean glutathione reductase (GR) value in 50 mM Taurine group was significantly ($P < 0.05$) higher at pre-freeze and post-thaw stages of cryopreservation as compared to that of the 25 mM Taurine, 75 mM Taurine and control groups.

Glutathione reductase (GR) values was found to be significantly ($P < 0.05$) lower in all the groups at pre-freeze stage as compared post-thaw stage of cryopreservation (Table 3).

These findings are in accordance with Perumal *et al.*, (2013) who have reported that the addition of 50 mM Taurine resulted in significant increase in Glutathione reductase

(GR) level but for the liquid storage (5°C) of Mithun bull (*Bos frontalis*) Semen.

However, Serpil *et al.*, (2009) have reported a significant decrease in Glutathione reductase (GR) level at 2 mM Taurine concentration in bull. Whereas, a non-significant effect of Taurine on Glutathione reductase (GR) levels in ram (Bucak *et al.*, 2007) has been reported.

The GR has a sustained ability in the maintenance of sperm membrane integrity and individual motility. So, higher the GR in the semen might be a factor in making the sperm membrane more resistant to the spontaneous lipid peroxidation that destroys the structure of the lipid matrix and is associated with the loss of motility (Perumal *et al.*, 2009).

Scavenging of the free radicals by Taurine, thereby alleviating GR consumption by the enzymatic antioxidant defenses (Huxtable, 1992) might be implicated in higher GR levels as observed in the present study or it may be due to its ability to maintain the antioxidant system and thereby increasing the level of the GR. (Bucak *et al.*, 2007).

In conclusions, The Malondialdehyde (MDA) level of neat semen of Gir bull which differed significantly between bulls. Whereas Glutathione reductase (GR) level of neat semen of did not differ significantly between bulls. Taurine (50 mM) as a semen additive when added to andromed extender, controls the oxidative stress as indicated by an increase in glutathione reductase and a decline in melondialdehyde (MDA) level at all the stages of cryopreservation in Gir bull semen addition of 50 mM Taurine as an additive in the and romed extender for cryopreservation of semen is most appropriate concentration in view to preserve and improve the semen quality in Gir bull semen.

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