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Evaluation of Antioxidant Potential of Taurine in Tris Egg Yolk Citrate Extender in Surti Buck Semen Preserved at Refrigerated Temperature

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ABSTRACT

Background: Taurine supplementation in tris egg yolk citrate (TEYC) extender can improve antioxidant defense and reduce motility degeneration rate in semen of Surti buck preserved at refrigeration temperature. Present study has evaluated antioxidant effect of different concentrations of taurine in TEYC extender on oxidative stress, antioxidant defense and motility degeneration rate in Surti buck semen preserved at refrigerated temperature.

Methods: Four Surti bucks (age>2years) were selected. Total 64 ejaculates (16 per buck) were collected during a period of 8 week. Post-collection they were pooled and stored at refrigeration temperature after dividing into 5 groups based on different taurine concentrations in TEYC extender *viz*. 0 mM (control T1), 25 mM (T2), 50 mM (T3), 75 mM (T4) and 100 mM (T5) taurine maintaining final concentration of 200 × 10^6 sperm/ml (pH 6.5-6.8). Evaluation of motility degeneration rate at 24, 36 and 48 hours and glutathione as well as lipid peroxidation (MDA) at 0 and 48 hours was done.

Result: Supplementation of taurine @50 mM in TEYC extender caused post-chilling significant increase in reduced glutathione (GSH) levels, lowering of lipid peroxidation (in terms of MDA production) and reduction of motility degeneration rate (MDR)% in semen of Surti bucks. It improved antioxidant defense thereby maintaining good quality of semen.

Key words: Antioxidant, Refrigerated, Surti buck semen, Taurine, TEYC.

INTRODUCTION

India is home to vast livestock resource that plays a significant role in improving socio-economic conditions in rural regions. About 20.5 million people depend upon livestock for their livelihood. Contribution of livestock sector amounts to 4.11% of total GDP and 25.6% of agriculture GDP of the country. Goat population in India in 2019 was 148.88 million that has shown 10.1% increase since Livestock Census 2012. Goat contributes 27.8% of the total livestock (20th Livestock Census). Goats are very easy to manage and they are versatile in quickly changing their capacity to adapt that is helpful in rearing them. However, their poor production potential necessitates genetic improvement by artificial insemination (AI) from superior sires that creates the need of preserving the semen maintaining its quality. Preservation of semen maintaining its quality acts as an important tool to conserve the valuable germplasm for improving the genetic quality of farm animals. Mammalian sperm membranes have many unsaturated fatty acids and are vulnerable to lipid peroxidation (LPO) in the presence of reactive oxygen species (ROS), leading to decreased sperm quality (Bucak *et al.,* 2007 and Lenzi *et al.,* 2002). The anti-oxidant system comprising glutathione peroxidase (GSH-PX), catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD) has been described as defense mechanisms that acts against the lipid peroxidation and is important in maintaining sperm motility and viability (Bilodeau *et al.,* 2001; Aitken and Baker, 2004 and Gadea *et al.,* 2004). Successful artificial insemination requires efficient freeze-thaw process of semen. Freezing may have deleterious effects. Therefore, cryoprotectant is added in cryopreservation extender to reduce the detrimental effects of the freezing process (Bucak *et al.,* 2007). This anti-oxidant capacity in sperms may however, be insufficient in preventing lipid peroxidation of sperm membrane during freeze-thawing process. To augment endogenous antioxidant defense some substance can be added in extenders for antioxidant effect. One such antioxidant is Taurine (2-aminoethanesulfonic acid) that can traverse sperm plasma membrane and inhibit lipid peroxidation,

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protect against accumulation of ROS (Chen *et al.,* 1993 and Singh *et al.,* 2012), modulate Ca^{++} uptake (Singh *et al.,* 2012) and inhibit protein phosphorylation (Kumar and Atreja, 2012). Considering the benefits of adding taurine in extender during cryopreservation of semen present study has been conducted to investigate the effect of different concentrations of taurine in tris egg yolk citrate semen extender on oxidative stress, antioxidant defense and motility degeneration rate of Surti buck semen preserved at refrigerated temperature.

MATERIALS AND METHODS

The present study was conducted at College of Veterinary Science and AH, Navsari Kamdhenu University (Gujarat, India) during the year 2021. Total four apparently healthy Surti bucks above two years of age maintained under All India Coordinated Research Project (AICRP) on goat at LRS, NAU, Navsari, Gujarat were selected. Selected bucks were managed under uniform managemental and feeding conditions. They were dewormed four times in a year and regularly vaccinated against common diseases *viz.* Peste des Petits Ruminants (PPR) and Foot and Mouth Disease (FMD). Bucks were trained for donating semen in artificial vagina by using female (doe) as dummy. After completion of the training period of about one month, semen was collected regularly by using artificial vagina twice a week from each buck for up to 8 weeks. Semen was collected from all selected bucks twice in a week at early morning between 6.30 AM to 7.30 AM with the help of eight-inch artificial vagina (AV) maintaining inner temperature of 40°C to 42°C and adequate pressure. Semen samples with motility ≥70% were considered for further processing. The TEYC diluter was prepared on the day of experiment by adding 20% egg yolk in Tris-citric acid-fructose buffer in sterile flask. The pooled semen was divided into five aliquots and each aliquot was diluted with extender containing Tris-egg yolk citrate diluter with 0 mM (control, T1), 25 mM (T2), 50 mM (T3), 75 mM (T4) and 100 mM (T5) taurine separately to set a final concentration of 200×10^6 sperms/ml (pH 6.5-6.8). Semen samples were examined for its physio-morphological parameters and parameters for oxidative stress and antioxidant defense during different hours of storage. Assessment of Motility degeneration rate (MDR) was done initially and 24, 36 and 48 hours post-chilling, Lipid peroxidation (LPO) and reduced glutathione (GSH) were assessed initially at 0 hour and thereafter at 48 hours post-chilling. Lipid peroxidation of spermatozoa was measured by determining the concentration of Malondialdehyde (MDA) production based on Thiobarbituric acid reaction (TBA) as an indicator for lipid peroxidation according to the method described by Buege and Aust (1978), Rao *et al.* (1989) and modified by Perumal *et al.* (2016) and Banday *et al.* (2017). Reduced glutathione (GSH) was estimated as per method described by Sedlak and Lindsay (1968). Data was analysed statistically by ANOVA using DMRT. Means were compared at P<0.05 and P<0.01 (Snedecor and Cochran, 1994). Correlation analysis was also done between different parameters.

RESULTS AND DISCUSSION

Mean values of motility degeneration rate (MDR) percentages, MDA levels for lipid peroxidation and GSH are mentioned in Table 1, 2 and 3 respectively. Correlation analysis among these parameters is presented in Table 4.

Motility degeneration rate (MDR)

Mean MDR per cent at 24, 36 and 48 hours was significantly (p<0.01) lower in T3 group (50 mM taurine) when compared to other concentrations T1 (0 mM), T2 (25 mM), T4 (75 mM) and T5 (100 mM) of taurine treated groups. A significantly increasing trend of MDR percent in different groups with increasing duration of storage was observed. Progressive increase in mean MDR percent with passage of time in the present study corroborated well with significantly (p<0.05) increased MDR at 12, 24, 36 and 48 hours intervals in the study of Amarjeet *et al.* (2019) in which they used pomegranate juice as an additive at different concentration in TEYC extender for cauda epididymal buck spermatozoa preserved at refrigeration temperature. Similarly Lima *et al.* (2013) also reported significantly (p<0.05) increased MDR at 12 and 48 hour intervals with different extenders on spermatozoa retrieved from six goat cauda epididymis when

Table 1: Effect of different concentrations of taurine on motility degeneration rate (MDR) percent (Mean±SE) of Surti buck semen at different storage duration preserved at refrigerated temperature.

Groups	MDR (%) (n=16)			F value	P value
	24 hours	36 hours	48 hours		
T1	$19.28\pm1.36^{b}_{y}$	$32.29\pm1.81^{b}_{x}$	$45.32\pm2.07^{b}_{w}$	54.91**	0.00
T2	$14.85\pm0.83^{c}_{y}$	$25.00\pm1.52^{c}_{x}$	$35.83\pm2.22^{c}_{w}$	41.64**	0.00
T3	$9.37\pm1.00^{d}_{y}$	$17.47\pm1.38^{d}_{x}$	$27.57\pm1.89^{d}_{w}$	38.43**	0.00
T4	$19.33\pm1.92^{b}_{y}$	$33.22\pm2.24^{b}_{x}$	$45.87\pm2.33^{b}_{w}$	37.52**	0.00
T5	$27.07\pm1.70^{a}_{y}$	$41.18\pm1.88^{a}_{x}$	$54.59\pm1.88^{a}_{w}$	57.08**	0.00
F value	21.28**	25.14**	24.83**	-	-
P value	0.00	0.00	0.00	-	-

$^{a-d}$Mean values with different superscript within a column (between the groups) differs significantly at p<0.01.

$_{w-y}$Mean values with different subscript between a column (between time intervals) differs significantly at p<0.01. **p<0.01.

Group/Concentration: T1 - Control, T2 - Taurine-25 mM, T3 - Taurine-50 mM, T4- Taurine-75 mM and T5 - Taurine-100 mM.

cooled at 4°C. An increasing trend of MDR percent with increase in preservation time as 30, 60 and 120 minutes was also seen in the study of Atara *et al.* (2019) during rainy and dry seasons in adult Surti buck semen when maintained at 37°C. However non-significant increase in MDR percent at 2, 24 and 48 hours intervals after cooling was found by Aguiar *et al*. (2013) during rainy season in non-defined breed of bucks. Sperm motility is an important criterion that should be sufficiently high for successful fertilization after AI. Under storage of semen sample oxidative stress increases that causes oxidative damage to spermatozoa resulting in higher motility degeneration rate. In terms MDR% T3 group (50 mM taurine) was better than others.

Lipid peroxidation (MDA production)

Initial mean MDA levels differed non-significantly at 0 hour between all the groups. Post-chilled mean MDA level at 48 hours was lower in T3 group (50 mM taurine) when compared to other concentrations T1 (0 mM taurine), T2 (25 mM), T4 (75 mM) and T5 (100 mM) of taurine treated groups. Further as compared to 0 hours with elapse of time, mean MDA levels of different groups were significantly ($p<0.01$) higher at 48 hours. These findings were in agreement with Perumal *et al.* (2013) who reported significantly ($p<0.05$) lower MDA ($nmol/10^8$ cells) production in 50 mM taurine supplemented group as compared to control group in tris egg yolk citrate extender during different hours of liquid storage (5°C) of Mithun bull semen. Likewise, Chhillar *et al.* (2012) reported significantly ($p<0.05$) lower MDA ($nmol/10^8$ cells) level in Karan Fries semen at post thawing with 50 mM taurine group in Tris-Egg Yolk Citrate (TEYC) extender as compared to control group. Atessahin *et al.* (2008) observed significant ($p<0.001$) lower MDA (nmol/ml) production in 75 mM taurine treated group and non-significant lower mean MDA production in 25 mM taurine treated group as compared to control group using Salomons Tris solution after freeze thawing in Angora goat semen. Supportive findings also emerge from the study of Banday *et al.* (2017) where they reported significantly ($p<0.05$) lower spermatic MDA ($nmol/10^8$ spermatozoa) production at post thaw stages of crossbred ram semen for 40 mM taurine group as compared to control in tris-based extender. However contrary to present findings, Bucak *et al.* (2007) observed that addition of 25 mM and 50 mM taurine to the tris-based extender did not cause any significant difference in MDA level as compared to the control group at post thawing stages. Sariozkan *et al.* (2009) also in contrast observed that addition of 2 mM taurine to Bioxcell® extender did not show any significant effect on

Table 2: Effect of different concentrations of taurine on lipid peroxidation (MDA) level (Mean±SE) of Surti buck semen preserved at different storage duration at refrigerated temperature.

Groups	MDA ($nmol/10^8$ sperm) (n=16)		F value	P value
	0 hour	48 hours		
T1	$3.63\pm0.08^{a}_{x}$	$6.15\pm0.42^{a}_{w}$	34.798**	0.00
T2	$3.26\pm0.15^{a}_{x}$	$5.23\pm0.30^{b}_{w}$	34.561**	0.00
T3	$3.45\pm0.32^{a}_{x}$	$4.67\pm0.16^{b}_{w}$	11.412**	0.00
T4	$3.37\pm0.21^{a}_{x}$	$5.08\pm0.14^{b}_{w}$	47.012**	0.00
T5	$3.64\pm0.20^{a}_{x}$	$6.06\pm0.24^{a}_{w}$	60.486**	0.00
F value	0.61	5.67**	-	-
P value	0.67	0.00	-	-

$^{a-b}$Mean values with different superscript within a column (between the groups) differs significantly at $p<0.01$.

$_{w-x}$Mean values with different subscript between a column (between time intervals) differs significantly at $p<0.01$. **$p<0.01$

Group/Concentration: T1 - Control , T2 - Taurine-25 mM, T3 - Taurine-50 mM, T4- Taurine-75 mM and T5 - Taurine-100 mM.

Table 3: Effect of different concentrations of taurine on reduced glutathione (GSH) level of Surti buck semen at different storage duration preserved at refrigerated temperature (Mean±SE).

Groups	GSH (nmol/ml) (n=16)		F value	P value
	0 hour	48 hours		
T1	$5.24\pm0.29^{a}_{w}$	$3.56\pm0.26^{ab}_{x}$	18.14**	0.000
T2	$4.83\pm0.27^{a}_{w}$	$3.81\pm0.18^{ab}_{x}$	10.24**	0.003
T3	$5.30\pm0.28^{a}_{w}$	$4.25\pm0.30^{a}_{x}$	6.55*	0.016
T4	$4.87\pm0.32^{a}_{w}$	$3.79\pm0.30^{ab}_{x}$	6.17*	0.019
T5	$5.21\pm0.21^{a}_{w}$	$3.46\pm0.16^{b}_{x}$	43.30**	0.000
F value	0.65	1.53*	-	-
P value	0.63	0.02	-	-

$^{a-b}$Mean values with different superscript within a column (between the groups) differs significantly at $p<0.05$, $p<0.01$.

$_{w-x}$Mean values with different subscript between a column (between time intervals) differs significantly at $p<0.05$, $p<0.01$. **$p<0.01$, *$p<0.05$.

Group/Concentration: T1 - Control , T2 - Taurine-25 mM, T3 - Taurine-50 mM, T4 - Taurine-75 mM and T5 - Taurine-100 mM.

Table 4: Correlation coefficients for oxidative stress parameters, antioxidant defense parameters and motility degeneration rate in Surti buck semen.

Traits	MDA 0 Hour	MDA 48 Hour	GSH 0 Hour	GSH 48 Hour	MDR 24 Hour	MDR 36 Hour	MDR 48 Hour
MDA 0 hour	1						
MDA 48 hour	0.203	1					
GSH 0 hour	0.032	.218	1				
GSH 48 hour	-0.066	.024	.560**	1			
MDR 24 hour	0.054	.255*	-.008	-.303**	1		
MDR 36 hour	0.083	.362**	.016	-.270*	.870**	1	
MDR 48 hour	0.093	.326**	.028	-.267*	.800**	.931**	1

*Correlation is significant at the ($p<0.05$) level.

**Correlation is significant at the ($p<0.01$) level.

lowering of MDA (nmol/ml) production following cryopreservation when compared to control group. Even though Mughal *et al.* (2013) also reported no effect on MDA production with supplementation of different concentrations of taurine (0, 20, 40, 60 mM) to the Lactose Egg yolk Glycerol Extender, they still observed that MDA production was minimum during cryopreservation at 20 mM concentration of taurine in buffalo bull semen. Hydroxyl radical inflicts damage to unsaturated fatty acids spermatozoa membrane that resulting in increased MDA levels. In the present study it was shown that T3 group (50 mM taurine) had least MDA levels.

Glutathione (GSH)

Initial mean GSH levels differed non-significantly at 0 hour between all the groups. Post-chilled mean GSH level at 48 hours was observed to be higher in T3 group (50 mM taurine) when compared to other concentrations T1 (0 mM taurine), T2 (25 mM), T4 (75 mM) and T5 (100 mM) of taurine treated groups. GSH level of different groups was significantly ($p<0.01$) lower at 48 hours as compared to 0 hours. In the present study, higher post-chilled mean GSH level in Surti buck semen was observed with 50 mM taurine concentration followed by 25 mM and 75 mM taurine concentration at 48 hours storage by using Tris Egg Yolk Citrate (TEYC) extender. This was in agreement with Perumal *et al.* (2013) where they reported significantly ($p<0.05$) higher level of GSH in 50 mM taurine treated group as compared to control group in tris egg yolk citrate extender during different hours of liquid storage (5°C) of Mithun bull semen. Contrary to present findings, Bucak *et al.* (2007) reported that addition of 25 mM and 50 mM taurine to the tris-based extender did not cause any significant difference in GSH level as compared to control group at post thawing stages. Likewise, Atessahin *et al.* (2008) reported that addition of 25 mM, 50 mM and 75 mM concentration of taurine to the Angora goat semen did not cause any significant difference in GSH levels at post thawing stages as compared to control group. Antioxidant GSH through Glutathione peroxidase (GSH-Px) converts H_2O_2 to H_2O and prevents formation of hydroxyl radical which otherwise would attack membrane unsaturated fatty acids. In the present study T3 group (50 mM taurine) had highest GSH levels.

Interrelationship of MDR, MDA and GSH

Correlation analysis shows that MDA and GSH at 48 hours post-chilling were significantly correlated to MDR% positively and negatively respectively. This can be understood by the antioxidant defense mechanism, which suggests that higher levels of GSH would minimize lipid peroxidation of membrane resulting in low MDA production thereby low MDR% and vice versa.

CONCLUSION

Supplementation of taurine in TEYC semen extender @ 50 mM for Surti buck semen preserved at refrigerated temperature increases GSH and reduces MDA thereby reducing MDR%. Thus, it improves antioxidant defense and helps in maintaining good quality of semen.

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Conflict of interest: None.

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