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INFLUENCE OF ANTIOXIDANT CYSTEINE AND TAURINE IN TRIS EXTENDER FOR REFRIGERATION PRESERVATION (5°C) OF SURTI BUFFALO SEMEN

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ABSTRACT
The study was undertaken on semen ejaculates of five Surti buffalo bulls (8 ejaculates per bull) by split sample technique. Immediately after initial evaluation, ejaculates were divided into five aliquots and extended at the concentration of 100 $\times 10^6$ spermatozoa ml^{-1} at 34°C with standard Tris-Fructose-Egg Yolk-Glycerol (TFYG) extender as control and TFYG having 2 additives - Cysteine HCl @ 0.5 & 1.0 mg/ml and Taurine @ 4.0 & 6.0 mg/ml - each at 2 levels, to study their comparative efficacy for refrigeration preservation (at 5°C up to 72 hrs), evaluated on 24 hourly interval, in terms of sperm quality parameters such as progressive motility, livability, total abnormalities, acrosomal integrity and plasma membrane integrity (HOS reactivity). The mean percentages of sperms soon on dilution (initial/0 hr) in different extender preparations viz. control TFYG, TFYG + cysteine @ 0.5 mg/ml, TFYG + cysteine @ 1.0 mg/ml, TFYG + taurine @ 4.0 mg/ml, TFYG + taurine @ 6 mg/ml varied significantly ($p<0.05$) in terms of progressive motility (75.12±0.76 to 81.75±0.79), livability (82.75 ±0.76 to 86.17±0.64), total abnormalities (4.60±0.24 to 4.93±0.20), acrosomal integrity (90.55±0.43 to 93.07±0.31) and HOS reactivity (82.72±0.91 to 86.25±0.64). Significant ($p<0.01$) variations among some of the above corresponding extender preparations were also observed after 24 hrs of refrigeration storage in terms of progressive motility (62.37±0.66, 65.37±0.76, 70.00±0.69, 69.00±0.82, 67.00±1.02), livability (82.75±0.76, 84.27±0.66, 85.95±0.65, 86.17±0.64, 84.80±0.82), total abnormalities (68.60 ±0.77, 72.05±0.92, 75.87±0.69, 75.05±0.93, 71.75±1.17), acrosomal integrity (89.75±0.31, 90.92±0.25, 91.72±0.32, 89.42±0.39, 87.45±0.34) and HOS reactivity (67.35±0.70, 71.17±0.90, 75.30±0.93, 74.37±1.00, 71.80±1.13); so also after 48 hrs of refrigeration and even after 72 hrs of refrigeration storage in respect of progressive motility (44.57±0.92, 48.87±0.93, 55.00±0.85, 55.00±0.82, 47.62 ±0.91), livability (50.47±1.03, 54.85±1.05, 60.50±0.85, 60.20±0.93, 53.95±1.01), total abnormalities (9.28±0.29, 8.30±0.25, 7.78±0.26, 8.23±0.31, 9.23±0.24), acrosomal integrity (83.10±0.33, 85.10±0.29, 86.32±0.31, 82.50±0.47, 79.75±0.39) and HOS reactivity (49.67±0.94, 53.40±0.94, 59.85±1.01, 59.00±0.82, 52.77±0.99). At all intervals of refrigeration storage (0-72 hrs) semen diluted with TFYG + cysteine @ 1.0 mg/ml and TFYG + taurine @ 4.0 mg/ml showed significantly ($p<0.05$) better sperm quality parameters than control TFYG, TFYG + cysteine @ 0.5 mg/ml and TFYG + taurine @ 6 mg/ml, and could sustain sperm quality in acceptable limit till 72 hrs. Thus cysteine 1.0 mg/ml or taurine 4.0 mg/ml in TFYG extender is considered as a better additive for refrigeration preservation of buffalo semen, yet *in vivo* fertility trials are warranted before recommending it for the routine use in semen extender by commercial semen banks.

KEY WORDS: Buffalo Semen, Refrigeration, Cysteine, Taurine, Sperm quality parameters.

INTRODUCTION

Buffalo semen is known for its poor keeping quality and freezability which is well documented in the literature (Roy *et al*., 1962; Sengupta *et al.*, 1963; Chaudhari *et al.*, 2015). The extender is an important factor which must have adequate pH and buffering capacity, appropriate osmolarity and should protect spermatozoa from cold shock. The Tris-Fructose-Egg Yolk-Glycerol (TFYG) extender is routinely used for bull semen preservation. Sperm plasma membrane is a primary target for damage or cold shock injury during preservation. It is suggested that higher susceptibility of mammalian spermatozoa towards oxidative stress may have been due to higher lipid peroxidation levels. Although bovine semen has natural defense system against the oxidative stress, it is considered insufficient under cryopreservation-mediated stress. Therefore, reinforcement of semen extender with suitable antioxidant is suggested to reduce oxidative damage during freeze-thawing of bull and buffalo spermatozoa (Ansari *et al*., 2011[a]). Mammalian spermatozoa membranes are very sensitive to free radical induced damage mediated by lipid peroxidation, as they are rich in polyunsaturated fatty acids. Limited endogenous mechanisms exist to reverse these damages. Reactive oxygen species (ROS) attacks the fluidity of the sperm plasma membrane and the integrity of DNA in the sperm nucleus. ROS induced DNA damage accelerate the germ cell apoptosis. Unfortunately spermatozoa are unable to

repair the damage induced by excessive ROS as they lack the cytoplasmic enzymes required to accomplish the repair. Hence, strategies must be directed toward lowering of ROS levels to keep only a small amount necessary to maintain normal cell function (Maneesh and Jayalekshmi, 2006). Motility as well as fertility of spermatozoa can be improved by incorporating various motility enhancing agents like pentoxifylline (PTX) or antioxidants. The antioxidants check the chemical breakdown of substrate resulting from oxidation. Antioxidant preservatives neutralize the free radicals that initiate and help propagate these reactions during storage and enhance the sperm survival (Strzeżek *et al*., 1999). Amino acids have important roles in preventing oxidative damage to spermatozoa during preservation. Cysteine, a precursor of intracellular glutathione, has been shown to penetrate the cell membrane easily, enhancing the intracellular GSH biosynthesis both *in vivo* and *in vitro* and protecting the membrane lipids and proteins due to indirect radical scavenging properties (Memon *et al*., 2011). Taurine is sulfonic amino acid and is non-enzymatic scavenger that plays an important role in protection of spermatozoa against ROS, in case of exposure to aerobic conditions and storage at 4°C under refrigerator (Perumal *et al.,* 2013). Keeping these facts in view, this study was aimed to find out the antioxidant effects of addition of the two amino acids -cysteine and taurine- at two different dose levels to the Tris-Egg Yolk-Glycerol extender for refrigeration preservation of buffalo semen.

MATERIALS & METHODS

The study was undertaken on five sexually mature healthy Surti buffalo bulls, aged 4 to 6 years, during the favourable breeding season at Central Sperm Station of the College in Anand, Gujarat (India). All these bulls were under veterinary care and were in regular twice a week semen collection schedule using artificial vagina. Eight ejaculates were studied from each bull (total 8 x 5 = 40 ejaculates) at weekly interval in a split-sample technique. The standard protocol as per FAO (1979) for preparation of TFYG extender was followed and fresh extender was prepared just before collection. Out of the 100 ml standard TFYG extender prepared, 5 aliquots of 20 ml each were made into different glass cylinders. Cysteine powder @ 10 mg, and 20 mg was added in two different cylinders containing 20 ml TFYG extender to give final concentration of 0.5 and 1.0 mg/ml, respectively. Similarly, taurine was added @ 80 mg and 120 mg to the 20 ml TFYG extender to give final concentration of 4.0 and 6.0 mg/ml, respectively. The fifth aliquot of 20 ml TFYG was kept as non-added control. Finally, prepared extenders were stirred with magnetic stirrer and kept in thermo-regulatory water bath at 34°C until used for extension. Immediately after collection the ejaculates were shifted in water bath at 34°C and evaluated for routine macroscopic and motility attributes. Only the ejaculates with >70% initial motility were further used for this study and were immediately diluted with different extender preparations @ 100 million sperms/ml and evaluated for sperm quality parameters such as motility, livability, morphology (eosin-nigrosin stain), acrosomal (Giemsa stain) and plasma membrane integrity (HOS test, 150 mOsm/l) as per standard procedures. The extended semen samples (at least 2 ml each) of different aliquots were then transferred to sugar tubes and placed in a refrigerator for gradual cooling and storage at 5°C. The individual sperm motility, viability, morphology, acrosomal integrity and plasma membrane integrity (HOST) were again assessed at 24 hourly intervals up to 72 hrs of preservation. The data generated were analyzed statistically using ANOVA and Duncan's new multiple range test by employing IBM SPSS Statistics version 20.00 to know the variation between different extender-additives and periods of preservation.

RESULTS & DISCUSSION

The comparative study on the effect of antioxidants (cysteine and taurine) for refrigeration (5°C) preservation of Surti buffalo semen in terms of sperm quality parameters such as motility, livability, morphology, acrosomal integrity and plasma membrane integrity (HOST) was carried out initially on dilution and then at 24 hourly intervals up to 72 hrs of refrigeration. The findings are presented in Tables 1-3. The results revealed that there were significant differences ($p<0.01$) in quality parameters between some of the semen extender preparations at a particular stage of preservation and between different stages of refrigeration storage also. In all the extender preparations with each increase in 24 hourly storage interval, the percentage of progressively motile sperms, live sperms and sperms with intact acrosome and HOS reactivity declined gradually and significantly ($P<0.01$) (Table 1), whereas the overall and segment-wise sperm abnormality as well as acrosomal abnormalities increased significantly ($P<0.05$) as the storage time increased (Table 2, 3). Extender TFYG with inclusion of cysteine 1 mg/ml and taurine 4 mg/ml, which were statistically similar, showed significantly better sperm progressive motility than TFYG without additives and even TFYG with cysteine 0.5 mg/ml and taurine 6 mg/ml, which were also statistically similar at most intervals of refrigeration preservation. The motility parameter showed somewhat conflicting results in TFYG with taurine 6 mg/ml, in which higher sperm motility was observed at initial hours of refrigeration storage, but it deteriorated at a higher rate at subsequent stages. The proportion of live sperm percentage in all the five extender treatments followed the trend of motile spermatozoa at each stage of refrigeration storage, the values in extender having cysteine 1 mg/ml and taurine 4 mg/ml were statistically higher than in other extender additives at all intervals after 24 hrs of preservation, followed by TFYG with taurine 6 mg/ml and cysteine 0.5 mg/ml, while the live sperm percentages in TFYG control extender were the lowest ($P<0.01$) than those with both the additives, irrespective of their level of inclusion (Table 1). The values of intact acrosome were highest in cysteine 1 mg/ml, followed by cysteine 0.5 mg/ml as compared to taurine 4 mg/ml, which was at par with control TFYG, while taurine at 6 mg/ml concentration in TFYG caused significant deterioration in acrosome quality even to that of non-added control TFYG at all intervals particularly at 48 hrs and 72 hrs of refrigeration preservation. The percentage of HOS reactive spermatozoa followed the trend near to that of intact

acrosome in all the five extender-additives at all the storage intervals. The highest HOS reactive sperm percentages were observed in TFYG with cysteine 1 mg/ml followed by taurine 4 mg/ml, while values for cysteine 0.5 mg/ml and taurine 6 mg/ml were lower and mostly statistically at par at all storage intervals, yet little better than control TFYG diluent. TFYG with cysteine 1 mg/ml proved to be the best membrane protector followed by TFYG with taurine 4 mg/ml, compared to other levels used, and all in fact better protected sperm plasma membrane over control during refrigeration preservation (Table 1).

TABLE 1: Mean (± SE) percentages of progressively motile, live, HOS reactive spermatozoa and intact acrosome in Surti buffalo bulls' semen at different intervals of refrigeration preservation (5˚C) in Tris extender without and with additives

Refrigeration Storage	Extender	Progressive motility %	Live sperm %	Intact acrosome %	HOS reactive sperm %
0-hr	TFYG Control	75.12^{a}±0.76	82.75^{a}±0.76	92.85^{b}±0.34	82.72^{a}±0.91
	Cysteine 0.5 mg/ml	77.75^{b}±0.69	84.27^{ab}±0.66	93.07^{b}±0.31	83.40^{a}±0.70
	Cysteine 1 mg/ml	80.51^{c}±0.65	85.95^{b}±0.65	91.00^{b}±2.30	85.72^{b}±0.67
	Taurine 4 mg/ml	81.75^{c}±0.79	86.17^{b}±0.64	92.22^{b}±0.39	86.25^{b}±0.64
	Taurine 6 mg/ml	81.00^{c}±0.82	84.80^{b}±0.82	90.55^{a}±0.43	85.62^{b}±0.86
	Pooled	**79.22^{s}±0.37**	**84.79^{s}±0.31**	**91.94^{s}±0.48**	**84.47^{s}±0.35**
24-hr	TFYG Control	62.37^{a}±0.66	68.60^{a}±0.77	89.75^{b}±0.31	67.35^{a}±0.70
	Cysteine 0.5 mg/ml	65.37^{b}±0.76	72.05^{b}±0.92	90.92^{c}±0.25	71.17^{b}±0.90
	Cysteine 1 mg/ml	70.00^{d}±0.69	75.87^{c}±0.69	91.72^{c}±0.32	75.30^{d}±0.93
	Taurine 4 mg/ml	69.00^{cd}±0.82	75.05^{c}±0.93	89.42^{b}±0.39	74.37^{cd}±1.00
	Taurine 6 mg/ml	67.00^{bc}±1.02	71.75^{b}±1.17	87.45^{a}±0.34	71.80^{bc}±1.13
	Pooled	**66.75^{r}±0.40**	**72.66^{r}±0.45**	**89.85^{r}±0.18**	**72.00^{r}±0.46**
48-hr	TFYG Control	53.50^{a}±0.82	59.17^{a}±0.93	86.72^{b}±0.29	57.52^{a}±0.82
	Cysteine 0.5 mg/ml	57.25^{b}±0.87	62.55^{b}±0.96	88.27^{c}±0.26	61.57^{b}±0.94
	Cysteine 1 mg/ml	61.75^{c}±0.85	67.85^{c}±0.85	89.05^{c}±0.32	67.30^{c}±0.96
	Taurine 4 mg/ml	60.75^{c}±0.79	66.37^{c}±0.99	86.10^{b}±0.33	65.32^{c}±0.80
	Taurine 6 mg/ml	56.87^{b}±0.95	61.70^{ab}±1.17	83.85^{a}±0.32	60.77^{b}±1.12
	Pooled	**58.02^{q}±0.43**	**63.53^{q}±0.48**	**86.80^{q}±0.18**	**62.50^{q}±0.48**
72-hr	TFYG Control	44.57^{a}±0.92	50.47^{a}±1.03	83.10^{b}±0.33	49.67^{a}±0.94
	Cysteine 0.5 mg/ml	48.87^{b}±0.93	54.85^{b}±1.05	85.10^{c}±0.29	53.40^{b}±0.94
	Cysteine 1 mg/ml	55.00^{c}±0.85	60.50^{c}±0.85	86.32^{d}±0.31	59.85^{c}±1.01
	Taurine 4 mg/ml	55.00^{c}±0.82	60.20^{c}±0.93	82.50^{b}±0.47	59.00^{c}±0.82
	Taurine 6 mg/ml	47.62^{b}±0.91	53.95^{b}±1.01	79.75^{a}±0.39	52.77^{b}±0.99
	Pooled	**50.20^{p}±0.49**	**55.99^{p}±0.51**	**83.55^{p}±0.22**	**54.95^{p}±0.50**

TFYG=Tris fructose yolk glycerol; Means bearing different superscripts between refrigeration storage intervals (p,q,r,s) and between additives (a,b,c,d,e) differ significantly ($P<0.05$).

Further, TFYG with 1 mg/ml cysteine and 4 mg/ml taurine showed lesser segment-wise as well as overall sperm abnormalities than other additives and control TFYG extender (Table 2). The total acrosomal abnormalities were the lowest in TFYG with 1 mg/ml cysteine followed by TFYG with 0.5 mg/ml cysteine and the highest acrosomal abnormalities were recorded in TFYG with taurine 6 mg/ml. The same trend was also observed for the incidence of each of the particular type of acrosomal abnormalities evaluated (Table 3). Our findings of sperm quality parameters evaluated at different refrigeration preservation intervals in Surti buffalo semen diluted with different extender additives particularly TFYG with cysteine 1 mg/ml corroborated well with many previous reports (Jaiswal *et al.*, 1988; Dhami and Sahni, 1993; Raval *et al.*, 2007). Cysteine is a low-molecular weight amino acid containing thiol which is a precursor of intracellular glutathione. It has been shown to penetrate the cell membrane easily, enhancing the intracellular GSH biosynthesis both *in vivo* and *in vitro* and protecting the

membrane lipids and proteins due to indirect radical scavenging properties. Study of Ansari *et al*. (2011[a,b]) about the supplementation of cysteine 0.5, 1.0, 2.0 and 3.0 mM in extender was in agreement to the present findings of improved protection of sperm quality of liquid semen by cysteine, but their observations of 0.5 mM being better than other levels and the quality deteriorated at above 1.00 mM concentration are contradictory with our results of superiority of cysteine 1 mg/ml than 0.5 mg/ml. On the contrary, Saxena *et al*. (1988) did not find any beneficial effect of glycine, cystiene hydrochloride or EDTA addition to either "Russian" diluent or egg yolk citrate, on preservability of bull semen at room and refrigeration temperature. This may be attributed to the difference in the basic diluents used in the study. In our study, taurine 4 mg/ml additions to TFYG extender lead to better preservation of most of the sperm quality parameters, except acrosome integrity. But these findings are in contradiction with the observations of Ae Oh *et al*. (2012), who reported that membrane integrity to swollen sperm ratio was significantly increased in taurine supplemented group. The studies by Kishore *et al*. (2011) had shown that semen fortification with taurine 20 mM resulted in significant and better quality preservation of most of the sperm parameters of bull semen.

TABLE 2: Mean (± SE) percentages of segment-wise and total sperm abnormalities in Surti buffalo bulls' semen at different intervals of refrigeration preservation (5°C) in Tris extender without and with additives

Refrigeration Storage	Extender	Sperm segments			Overall
		Head	Midpiece	Tail	
0-hr	TFYG Control	1.20±0.89	1.60±0.10	2.12±0.19	4.93±0.25
	Cysteine 0.5 mg/ml	1.20±0.89	1.55±0.10	1.90±0.18	4.68±0.26
	Cysteine 1 mg/ml	1.22±0.11	1.42±0.09	1.92±0.19	4.92±2.20
	Taurine 4 mg/ml	1.17±0.08	1.47±0.11	1.95±0.18	4.60±0.24
	Taurine 6 mg/ml	1.20±0.07	1.62±0.09	2.10±0.17	4.93±0.20
	Pooled	**1.20^{p}±0.04**	**1.53^{p}±0.04**	**2.00^{p}±0.08**	**5.17^{p}±0.45**
24-hr	TFYG Control	1.25±0.07	1.87±0.12	3.00^{b}±0.16	6.13^{c}±0.24
	Cysteine 0.5 mg/ml	1.12±0.08	1.87±0.09	2.50^{ab}±0.17	5.50^{ab}±0.21
	Cysteine 1 mg/ml	1.25±0.09	1.77±0.11	2.35^{a}±0.18	5.35^{a}±0.24
	Taurine 4 mg/ml	1.12±0.08	1.95±0.11	2.70^{ab}±0.20	5.78^{ab}±0.28
	Taurine 6 mg/ml	1.27±0.07	1.95±0.11	2.97^{b}±0.17	6.20^{c}±0.23
	Pooled	**1.20^{p}±0.37**	**1.88^{q}±0.49**	**2.70^{q}±0.82**	**5.79^{q}±0.11**
48-hr	TFYG Control	1.37±0.08	2.37±0.14	3.75^{b}±0.14	7.50^{bc}±0.27
	Cysteine 0.5 mg/ml	1.27±0.08	2.15±0.12	3.25^{ab}±09.17	6.68^{a}±0.25
	Cysteine 1 mg/ml	1.32±0.08	2.30±0.11	2.82^{a}±0.16	6.45^{a}±0.27
	Taurine 4 mg/ml	1.30±0.18	2.32±0.29	3.27^{b}±0.53	6.93^{ab}±0.30
	Taurine 6 mg/ml	1.50±0.80	2.50±0.13	3.70^{ab}±0.16	7.70^{c}±0.22
	Pooled	**1.35^{q}±0.03**	**2.33^{r}±0.49**	**3.36^{r}±0.08**	**7.05^{r}±0.12**
72-hr	TFYG Control	1.52±0.07	3.02^{c}±0.14	4.72^{c}±0.17	9.28^{b}±0.29
	Cysteine 0.5 mg/ml	1.55±0.07	2.65^{ab}±0.13	4.12^{ab}±0.16	8.30^{a}±0.25
	Cysteine 1 mg/ml	1.50±0.08	2.55^{a}±0.12	3.75^{a}±0.17	7.78^{a}±0.26
	Taurine 4 mg/ml	1.52±0.08	2.77^{ab}±0.14	3.92^{a}±0.19	8.23^{a}±0.31
	Taurine 6 mg/ml	1.65±0.08	2.97^{b}±0.11	4.60^{bc}±0.17	9.23^{b}±0.24
	Pooled	**1.55^{r}±0.03**	**2.79^{s}±0.06**	**4.22^{s}±0.08**	**8.56^{s}±0.13**

TFYG=Tris fructose yolk glycerol; Means bearing different superscripts between refrigeration intervals (p,q,r,s) and between additives (a,b,c,d,e) differ significantly (P<0.05).

Our findings are in accordance with their observations that supplementation of taurine resulted in significantly better semen quality parameters after refrigeration preservation. Taurine is an intracellular amino acid found in majority of the mammalian tissues and plays its role in cell proliferation, viability, osmo-regulation and prevents injuries induced by oxidants in many tissues. It also maintains the stability of bio-membranes, scavenges ROS,

minimizes the end products of lipid peroxidation, modulates Ca2+ uptake and inhibits protein phosphorylation (Kumar and Atreja, 2012). Hence, the improved semen preservability observed after addition of taurine at certain levels in semen extender can be attributed to these functions of taurine, which is mainly through scavenging of reactive oxygen species.

TABLE 3: Mean (± SE) percentages of various types of sperm acrosomal abnormalities in Surti buffalo bulls' semen at different intervals of refrigeration preservation (5 °C) in Tris extender without and with additives

Refrigeration Storage	Extender	Sperm Acrosomal Abnormalities				Overall
		Swollen	Ruffled	Detached	Denuded	
0-hr	TFYG	3.02^{a}±0.15	2.17^{a}±0.12	1.32^{ab}±0.12	0.68^{a}±0.08	7.15^{b}±0.34
	C1	2.95^{a}±0.16	2.15^{a}±0.11	1.18^{a}±0.07	0.70^{a}±0.09	6.93^{a}±0.32
	C2	2.98^{a}±0.19	2.05^{a}±0.12	1.20^{a}±0.07	0.65^{a}±0.08	8.88^{b}±2.18
	T1	3.20^{a}±0.17	2.30^{a}±0.13	1.45^{b}±0.09	0.80^{b}±0.08	7.78^{b}±0.39
	T2	3.83^{b}±0.18	2.85^{b}±0.16	1.68^{c}±0.10	0.98^{b}±0.10	9.33^{b}±0.45
	Av.	**3.20^{p}±0.08**	**2.31^{p}±0.06**	**1.37^{p}±0.04**	**0.76^{p}±0.04**	**8.01^{p}±0.46**
24-hr	TFYG	4.55^{b}±0.17	3.10^{c}±0.12	1.65^{a}±0.08	0.95^{a}±0.06	10.25^{b}±0.31
	C1	3.85^{a}±0.12	2.75^{b}±0.11	1.58^{a}±0.09	0.88^{a}±0.06	9.08^{a}±0.29
	C2	3.53^{a}±0.12	2.35^{a}±0.10	1.48^{a}±0.09	0.93^{a}±0.15	8.28^{a}±0.32
	T1	4.58^{b}±0.17	3.28^{c}±0.16	1.73^{a}±0.09	1.00^{ab}±0.09	10.58^{b}±0.40
	T2	5.33^{c}±0.14	3.88^{d}±0.13	2.13^{b}±0.11	1.25^{b}±0.10	12.55^{c}±0.35
	Av.	**4.37^{q}±0.08**	**3.07^{q}±0.07**	**1.71^{q}±0.04**	**1.00^{q}±0.04**	**10.15^{q}±0.18**
48-hr	TFYG	5.78^{b}±0.14	4.35^{b}±0.15	1.98^{a}±0.10	1.18^{a}±0.07	13.28^{b}±0.30
	C1	5.00^{a}±0.14	3.55^{a}±0.11	2.00^{a}±0.07	1.18^{a}±0.06	11.73^{a}±0.27
	C2	4.68^{a}±0.17	3.33^{a}±0.14	1.83^{a}±0.09	1.13^{a}±0.06	10.95^{a}±0.33
	T1	5.68^{b}±0.16	4.33^{b}±0.13	2.40^{b}±0.09	1.48^{b}±0.09	13.90^{b}±0.34
	T2	6.48^{c}±0.13	5.00^{c}±0.15	2.78^{c}±0.11	1.83^{c}±0.10	16.15^{c}±0.33
	Av.	**5.52^{r}±0.08**	**4.11^{r}±0.07**	**2.20^{r}±0.05**	**1.36^{r}±0.04**	**13.20^{r}±0.19**
72-hr	TFYG	6.65^{bc}±0.14	5.48^{c}±0.15	2.93^{b}±0.12	1.85^{a}±0.10	16.90^{c}±0.33
	C1	6.28^{b}±0.13	4.65^{b}±0.14	2.45^{a}±0.11	1.50^{a}±0.12	14.88^{b}±0.30
	C2	5.68^{a}±0.13	4.20^{a}±0.15	2.30^{a}±0.10	1.50^{a}±0.09	13.68^{a}±0.32
	T1	6.85^{c}±0.17	5.45^{c}±0.18	2.95^{b}±0.10	2.23^{b}±0.18	17.50^{c}±0.48
	T2	7.70^{d}±0.15	6.55^{d}±0.17	3.63^{c}±0.12	2.35^{b}±0.10	20.25^{d}±0.39
	Av.	**6.63^{s}±0.08**	**5.27^{s}±0.09**	**2.85^{s}±0.06**	**1.89^{s}±0.06**	**16.64^{s}±0.23**

TFYG=Tris fructose yolk glycerol; C1= TFYG+Cysteine 0.5 mg/ml; C2=TFYG+Cysteine 1 mg/ml; T1=TFYG+taurine 4 mg/ml; T2=TFYG+taurine 6 mg/ml. Means bearing different superscripts between refrigeration intervals (p, q, r, s) and between additives (a, b, c, d, e) differ significantly (P<0.05).

CONCLUSION

Significantly (p<0.05) higher progressive sperm motility, with better viability and lesser abnormalities of buffalo spermatozoa were observed at all intervals of refrigeration storage (0-72 hrs) in semen diluted with standard TFYG extender having cysteine 1 mg/ml or taurine 4 mg/ml as additive than with 0.5 mg/ml cysteine and 6 mg/ml taurine and both the additives maintained significantly better sperm quality when compared with non-added control TFYG diluents. Taurine 6 mg/ml at times revealed insignificant differences with control TFYG for some sperm parameters. Therefore, TFYG extender with cysteine 1 mg/ml or taurine 4 mg/ml is considered as a better additive for refrigeration preservation of buffalo semen. However, *in vivo* fertility trials need to be conducted to validate and recommend routine supplementation of L-cysteine at 1 mg/ml or taurine at 4 mg/ml in TFYG extender for improved refrigeration of buffalo semen.

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