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Inclusion of Sericin in Tris Egg Yolk Extender Improves Bovine Sperm Quality during Cryopreservation (-196°C)

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Abstract

A study was undertaken on semen of 3 Gir and 3 Murrah bulls to assess the effect of different concentration of antioxidant Sericin (0.10, 0.25, 0.50 and 1.00 %, w/v) in tris fructose yolk glycerol (TFYG) extender for improving its cryopreservation. Ejaculates with >70% initial motility were split- diluted @ 100x106 sperm ml-1 with extender without and with Sericin, and were soon evaluated for sperm quality parameters, viz., progressive sperm motility, morphology, viability, intact acrosome and HOS test. The split-diluted samples were then filled in French mini straws, equilibrated for 4 hrs at 4 ℃ and frozen in liquid nitrogen vapour using a programmable bio-freezer. Straws thawed were again evaluated for post-thaw quality parameters including post-thaw incubation survival at 37 ℃ for 3 hr in water bath. The mean progressively motile spermatozoa observed, irrespective of Sericin levels, on dilution, at pre-freeze and post-thaw stage in Gir bull semen were 76.93±0.39, 59.90±0.47 and 43.47±0.58 %, and in Murrah bull 78.20±0.38, 60.67±0.43 and 44.10±0.48 %, respectively. The mean values on dilution and at post-thaw stage for live sperm were 80.19±0.32 and 48.41±0.59 % in Gir, and 81.08±0.32 and 48.59±0.46 % in Murrah bull semen; total abnormal sperm 6.34±0.16 and 11.55±0.14 % in Gir and 6.64±0.12 and 11.27±0.13 % in Murrah bull semen; intact acrosome 89.86±0.20 and 82.58±0.23 % in Gir and 92.30±0.19 and 85.13±0.18 % in Murrah, and HOS reactive sperm 71.19±0.45 and 39.56±0.57 % in Gir and 71.62±0.42 and 39.33±0.54 % in Murrah bull semen, respectively. The mean progressively motile sperm observed after 1, 2 and 3 hrs of post-thaw incubation of semen at 37 ℃ were 30.13±0.48, 19.63±0.42 and 10.77±0.40 % ($p<0.001$) in Gir, and 30.67±0.39, 20.70±0.37 and 12.20±0.31 % ($p<0.001$) in Murrah bull semen, respectively. The mean values of all above traits observed on dilution and at post-thaw stage/post-thaw incubation were significantly ($p<0.05$) better in TFYG extender with 0.50% and/or 0.25% Sericin than 0.10 or 1.00 % Sericin and control dilutor for both Gir and Murrah bull semen. It is concluded that inclusion of Sericin at 0.5% w/v in TFYG extender significantly improves post-thaw quality of bovine semen.

Keywords: Antioxidant Effect, Bovine Semen, Cryopreservation, Sericin, Sperm Quality, Post-Thaw Longevity

Introduction

Artificial insemination is the first-generation biotechnological advancement that has made a profound contribution to the genetic improvement, mainly in dairy bulls through which a single ejaculate from male is used to impregnate many females, particularly with its cryopreserved semen. Various steps of cryopreservation such as processing, freezing and thawing, however, exert physical, physiological as well as chemical stress on the sperm membrane, associated with an oxidative stress induced by free radicals. Naturally occurring antioxidants in semen protect the acrosomal integrity of the spermatozoa by reducing levels of reactive oxygen species (ROS) molecules and lipid peroxidation of cell membrane (Cotran *et al.,* 1989). The freeze thawing process decreases the population of sperm with intact acrosomes (Rasul *et al.,* 2001; Kumar *et al.,* 2011); decreases the antioxidant potential of the semen during cryopreservation and increases the lipid peroxidation levels and ROS molecules (Kadirvel *et al.,* 2009, Kumar *et al.,* 2011; Patel *et al.,* 2019). Sperm cells have a high content of unsaturated fatty acids in their membrane, but lack in a significant cytoplasmic component containing antioxidants. Hence, they are highly susceptible to lipid peroxidation by oxygen free radicals and H_2O_2. So, a variety of additives like antioxidants, membrane stabilizers, motility enhancers and chelating agents have been used to protect spermatozoa from deleterious effects of cryopreservation and for improving freezability and fertility of bull semen. Sericin, a silkworm protein, is an important molecule currently being used in cancer therapy for its polarity and high antioxidant antifreeze potential and chemical structure to produce required materials with improved property. The oocyte culture medium supplemented @ 0.1% sericin improved fertilizing ability of sheep oocytes (Yasmin *et al.,* 2015). Recently sericin has also been indicated to improve quality of frozen-thawed semen through protecting sperms from oxidative stress (Kumar *et al.,* 2015; Demra *et al.,* 2017). Furthermore, due to the reduced technological inventions on semen cryopreservation over the last few years (Celeghini *et al.,* 2008), the tris-egg yolk-fructose-glycerol extender is still the most frequently employed semen extender worldwide (Chaudhari *et al.,* 2015). The aim of this study was to assess the effect of different levels of sericin as antioxidant in tris egg yolk glycerol extender on sperm quality during cryopreservation (-196ºC) of Gir cattle and Murrah buffalo bull semen.

Materials and Methods

This investigation was carried out during September to February months of year 2018-19 on semen of three mature bulls (5-8 years old) each of Gir cattle and Murrah buffalo breeds maintained at Sperm Station of the College of Veterinary Science & AH, AAU, Anand. The bulls were maintained under identical nutritional and managerial conditions. The semen was collected twice a week from all the bulls in the morning hours using artificial vagina. For this study, alternate ejaculates were used under split-sample technique to evaluate effect of different levels of sericin as antioxidant in tris fructose yolk glycerol (TFYG) extender on sperm quality during cryopreservation through various morphological and functional attributes of spermatozoa. The ejaculates (10/bull, total 60) with >75 % initial motility were divided in to five equal aliquots, and extended at the concentration of 100×10^6 spermatozoa ml^{-1} at 34°C with TFYG extender without (control) and with sericin (Silkworm protein, Sigma-Aldrich, USA) @ 0.10, 0.25, 0.50 and 1.00 % w/v.

The extended semen samples were evaluated for sperm quality parameters, *viz.*, motility, viability, morphology, acrosomal integrity and plasma membrane integrity (HOST) through standard procedures and were filled in French mini straws using IS4 system (IMV, France). After gradual cooling over 60-90 minutes and equilibration for 4 hrs in cold handling cabinet, the straws were frozen in liquid nitrogen vapour using a previously calibrated programmable bio-freezer (IMV, France) for bovine semen. Just before freezing (after equilibration) only the pre-freeze motility was evaluated. The frozen stored straws were thawed next day in water bath at 37°C for 30 seconds and again evaluated for all above sperm quality parameters. Post-thaw incubation test (37 ºC) was performed to evaluate post-thaw longevity/sperm survival by examining the percentage of progressive motile sperm at 1, 2 and 3 hr of incubation. The data obtained were analyzed statistically using CRD and DNMRT within the breed, and by 't' test between breeds at each level by employing IBM SPSS software version 20 (IBM India Pvt Ltd, Bengaluru, India).

Results and Discussion

The mean percentages of progressively motile, live, abnormal spermatozoa, intact acrosome and HOS reactive spermatozoa observed on dilution and at post-thaw stage of Gir and Murrah bull semen extended in conventional standard tris fructose egg-yolk glycerol extender (TFYG) as control and TFYG added with antioxidant Sericin at

the concentration of 0.10, 0.25, 0.50 and 1.00 % (w/v) are presented in Tables 1-3. Statistical analysis revealed that there were highly significant differences ($p<0.01$) in all these parameters between different levels of Sericin and between stages of cryopreservation.

Progressive Sperm Motility and Viability

The overall mean percentages of progressively motile spermatozoa observed initially (on dilution), after equilibration (pre-freeze stage) and after freezing-thawing (post-thaw stage) of Gir bull semen in TFYG extender, irrespective of levels of Sericin (0.1 to 1.0% w/v), were 76.93±0.39, 59.90±0.47 and 43.47±0.58, respectively, and for Murrah bull semen 78.20 ±0.38, 60.67±0.43 and 44.10±0.48, respectively (Table 1).

Table 1: Mean (±SE) percentages of progressively motile spermatozoa in Gir cattle and Murrah buffalo bulls' semen during cryopreservation process and post-thaw incubation at 37°C in Tris extender containing different levels of sericin

Freezing stage	Sericin Levels in TFYG Extender	Progressive motile sperm (%)		Post-thaw incubation (hr) at 37°C	Post-thaw longevity (%)	
		Gir bulls	Murrah bulls		Gir bulls	Murrah bulls
On dilution	Seri 0.00%	74.50±0.55^{a}	76.00±0.44^{a}	1 hr	29.33±0.98^{ab}	29.17±0.64^{ab}
	Seri 0.10%	76.17±0.78^{ab}	77.00±0.82^{a}		29.50±1.08^{ab}	30.50±0.77^{ab}
	Seri 0.25%	77.83±0.75^{b}	77.83±0.92^{ab}		31.33±0.99^{ab}	31.33±0.76^{b}
	Seri 0.50%	80.50±0.77^{c}	80.83±0.76^{c}		32.17±0.98^{b}	33.67±0.86^{c}
	Seri 1.00%	75.67±1.04^{ab}	79.33±0.92^{bc}		28.33±1.28^{a}	28.67±1.04^{a}
	Average	76.93±0.39^{x}	78.20±0.38 ^{x}		30.13±0.48	30.67±0.39
Pre-freeze	Seri 0.00%	58.33±1.00^{a}	58.83±0.89^{a}	2 hr	18.50±0.76^{ab}	19.00±0.65^{a}
	Seri 0.10%	59.33±1.01^{ab}	60.67±0.92^{ab}		19.67±0.89^{abc}	20.50±0.69^{a}
	Seri 0.25%	61.17±0.95^{bc}	60.67±0.86^{ab}		20.67±0.98^{bc}	21.17±0.78^{a}
	Seri 0.50%	62.33±1.12^{c}	62.83±0.98^{b}		21.67±0.84^{c}	23.83±0.66^{b}
	Seri 1.00%	58.33±0.47^{a}	60.33±1.04^{ab}		17.67±1.01^{a}	19.00±1.00^{a}
	Average	59.90±0.47^{y}	60.67±0.43^{y}		19.63±0.42	20.70±0.37
Post-thaw (0 hr)	Seri 0.00%	42.17±1.12^{ab}	42.33±1.01^{a}	3 hr	10.33±0.76	10.83±0.48^{a}
	Seri 0.10%	42.67±1.29^{ab}	43.67±0.93^{a}		10.17±0.91	12.50±0.52^{a}
	Seri 0.25%	45.50±1.03^{bc}	44.83±0.81^{ab}		11.17±0.78	12.00±0.66^{a}
	Seri 0.50%	46.33±1.24^{c}	47.33±1.01^{b}		12.33±0.92	14.50±0.69^{b}
	Seri 1.00%	40.67±1.51^{a}	42.33±1.37^{a}		09.83±1.03	11.17±0.85^{a}
	Average	43.47±0.58^{z}	44.10±0.48^{z}		10.77±0.40	12.20±0.31

Means bearing different superscripts between sericin levels (abc) at each stage, and between stages (xyz) differ significantly ($p<0.05$)

The mean percentages of live spermatozoa observed on dilution and at post-thaw stage were 80.19±0.32 and 48.41±0.59 in Gir, and 81.08±0.32 and 48.59±0.46 in Murrah bull semen, respectively (Table 2). The values of both the traits differed highly significantly ($p<0.01$) between stages in both the breeds, but not between breeds at any of the stages. The values for sperm motility and viability were significantly ($p<0.05$) higher in extender containing sericin at the level of 0.50% and/or 0.25 % sericin as compared to higher or lower levels of sericin and control extender in both the breeds at all three stages (Tables 1, 2). The bull to bull variation was also significant for both the traits at post-thaw stage in both the breeds. This beneficial effect of sericin was also substantiated by significantly reduced lipid peroxidation (MDA production) and higher concentration of superoxide dismutase and glutathione peroxidase in the seminal plasma with increasing concentration of Sericin (Patel *et al.*, 2019).

Table 2: Mean (±SE) percentages of Live, Acrosome intact and HOS reactive spermatozoa in Gir cattle and Murrah buffalo bulls' semen during cryopreservation process in Tris extender containing different levels of sericin

Freezing stage	Sericin Levels in Extender	Live Sperm (%)		Intact Acrosome (%)		HOS Reactive Sperm (%)	
		Gir bulls	Murrah bulls	Gir bulls	Murrah bulls	Gir bulls	Murrah bulls
On dilution	0.00%	77.97±0.50^{a}	79.07±0.39^{a}	88.87±0.47^{c}	91.47±0.41^{b}	68.27±0.84^{a}	69.30±0.69^{a}
	0.10%	80.00±0.61^{bc}	79.67±0.68^{ab}	89.67±0.44^{bc}	92.43±0.39^{ab}	70.27±0.86^{ab}	70.17±0.97^{ab}
	0.25%	81.00±0.58^{c}	81.07±0.84^{bc}	90.50±0.39^{ab}	92.37±0.44^{ab}	72.70±0.77^{bc}	71.63±0.94^{ab}
	0.50%	83.30±0.58^{d}	83.40±0.59^{d}	91.10±0.35^{a}	93.00±0.43^{a}	75.17±0.84^{c}	74.37±0.75^{c}
	1.00%	78.67±0.85^{ab}	82.20±0.78^{cd}	89.17±0.20^{c}	92.23±0.44^{ab}	69.57±1.21^{a}	72.63±1.07^{bc}
	Average	80.19±0.32^{x}	81.08±0.32 ^{x}	89.86±0.20^{x}	92.30±0.19^{x}	71.19±0.45^{x}	71.62±0.42^{x}
Post-thaw	0.00%	46.63±1.17^{ab}	46.73±0.95^{a}	80.90±0.48^{c}	83.37±0.36^{c}	37.63±1.16^{a}	37.37±1.10^{a}
	0.10%	47.33±1.28^{ab}	48.30±0.81^{a}	82.53±0.48^{ab}	85.10±0.31^{b}	39.37±1.14^{ab}	39.00±1.11^{a}
	0.25%	50.13±1.13^{bc}	49.30±0.84^{ab}	83.93±0.30^{a}	85.77±0.33^{ab}	41.13±1.09^{b}	39.83±0.96^{a}
	0.50%	51.73±1.15^{c}	51.47±0.91^{b}	83.67±0.48^{a}	86.27±0.40^{a}	42.23±1.37^{b}	42.83±1.19^{b}
	1.00%	46.23±1.57^{a}	47.13±1.37^{a}	81.87±0.62^{bc}	85.13±0.47^{b}	37.43±1.46^{a}	37.63±1.40^{a}
	Average	48.41±0.59^{y}	48.59±0.46^{y}	82.58±0.23^{y}	85.13±0.18^{y}	39.56±0.57^{y}	39.33±0.54^{y}

Means bearing different superscripts between sericin levels (abc) at each stage, and between stages (xyz) differ significantly ($p<0.05$)

These findings on sperm motility concurred well with the observations of Dorji *et al.* (2015) and Kumar *et al.* (2015) in Thai bull and buffalo bulls, while Demra *et al.* (2017) found non-significantly higher ($p>0.05$) post-thaw sperm motility and viability with 0.25 % sericin supplementation as compared to control TFYG extender or with supplementation of 0.5, 0.75 and 1.0 % sericin, the higher level in fact was found detrimental. Patel *et al.* (2016) reported higher values than our finding of live sperm per cent on dilution in either of the breeds. At post-thaw stage, Beheshti *et al.* (2013), Patel *et al.* (2016) and Demra *et al.* (2017) revealed higher sperm viability than our findings, but Dorji *et al.* (2015) found relatively lower values in Thai bulls. Several other studies (Dhami *et al.*, 1994, Sariozkan *et al.*, 2009; Chhillar *et al.*, 2012; Patel *et al.*, 2016; Kurmi *et al.*, 2018) also favoured inclusion of various antioxidants such as cysteine, taurine, trehalose, vitamin E, ascorbic acid, glutathione at certain optimal levels in the semen extenders for improving significantly the sperm motility/ viability and cryopreservability of bovine semen by reducing ROS production and cryoinjury to the spermatozoa.

Sperm Abnormalities

The overall mean percentages of morphologically abnormal total spermatozoa observed initially on dilution, and at post-thaw stage in Gir bull semen, irrespective of Sericin levels, were 6.34±0.16 and 11.55±0.14, and for Murrah bull semen 6.64±0.12 and 11.27 ±0.13, respectively. These values differed significantly ($p<0.01$) between stages in both the breeds, but not between breeds at any of the stages (Table 3). The values were significantly lower at both the stages for both the breeds in TFYG with 0.50 and/or 0.25 % sericin compared to other levels or control extender. The bull effect was significant for total sperm abnormalities on dilution in both the breeds and at post-thaw stage in Gir breed only.The overall mean values of sperms with head, mid-piece and tail abnormalities recorded initially on dilution of Gir bull semen, irrespective of Sericin levels, were 2.05±0.05, 0.77±0.04 and 3.65±0.09 %, respectively. The corresponding values noted for Murrah bull semen were 2.19±0.05, 0.83±0.04 and 3.62±0.08 %. The respective mean values at post-thaw stage were 2.95±0.05, 1.17±0.05 and 7.43±0.11 % in Gir bulls, and 3.20±0.05, 1.27±0.04 and 6.79 ±0.10 % in Murrah bulls, respectively. The percentages of segment-wise sperm abnormalities increased highly significantly ($p<0.01$) at post-thaw stage over initial values in both the breeds. Moreover, the trend of segment-wise sperm abnormalities followed the overall trend of total sperm abnormalities between different levels of antioxidant sericin at both the steps of cryopreservation of semen, 0.50 and/or 0.25 % being superior in preventing total and segmental sperm abnormalities over other levels and control TFYG extender in both cattle and buffalo semen (Table 3).

Table 3: Mean (± SE) percentages of segment wise sperm abnormalities in Gir cattle and Murrah buffalo bulls' semen on dilution and after freezing-thawing in Tris extender containing different levels of sericin

Freezing Stage	Sericin Levels in TFYG Extender	Gir Cattle				Murrah Buffalo			
		Sperm Abnormalities				Sperm Abnormalities			
		Head	Mid-piece	Tail	Total	Head	Mid-piece	Tail	Total
On dilution	Seri 0.00%	2.20±0.07^{c}	1.03±0.06^{c}	3.87±0.20	**7.10±0.22^{b}**	2.40±0.10^{c}	0.93±0.08^{b}	3.83±0.17	**7.17±0.26^{c}**
	Seri 0.10%	2.13±0.09^{bc}	0.80±0.09^{bc}	3.63±0.19	**6.57±0.22^{b}**	2.40±0.09^{c}	0.93±0.08^{b}	3.53±0.16	**6.87±0.21^{bc}**
	Seri 0.25%	1.90±0.12^{ab}	0.50±0.09^{a}	3.50±0.19	**5.90±0.25^{a}**	2.03±0.12^{ab}	0.73±0.08^{ab}	3.50±0.17	**6.27±0.24^{ab}**
	Seri 0.50%	1.73±0.08^{a}	0.57±0.12^{ab}	3.50±0.22	**5.80±0.25^{a}**	1.87±0.10^{a}	0.67±0.09^{a}	3.43±0.16	**5.97±0.27^{a}**
	Seri 1.00%	2.30±0.10^{c}	0.93±0.08^{c}	3.77±0.18	**7.00±0.22^{b}**	2.23±0.12^{bc}	0.90±0.07^{ab}	3.80±0.20	**6.93±0.29^{bc}**
	Average	**2.05±0.05^{qx}**	**0.77±0.04^{px}**	**3.65±0.09^{rx}**	**6.47±0.11^{x}**	**2.19±0.05^{qx}**	**0.83±0.04^{px}**	**3.62±0.08^{rx}**	**6.64±0.12^{x}**
Post-thaw	Seri 0.00%	3.10±0.07^{bc}	1.30±0.09^{bc}	7.97±0.22^{c}	**12.37±0.27^{b}**	3.50±0.09^{c}	1.37±0.09^{b}	7.53±0.14^{c}	**12.40±0.19^{c}**
	Seri 0.10%	3.10±0.10^{bc}	1.20±0.11^{abc}	7.40±0.21^{abc}	**11.70±0.26^{b}**	3.20±0.09^{b}	1.30±0.09^{b}	6.77±0.22^{ab}	**11.27±0.28^{b}**
	Seri 0.25%	2.60±0.11^{a}	1.03±0.11^{ab}	6.93±0.21^{a}	**10.57±0.27^{a}**	3.13±0.10^{ab}	1.23±0.08^{ab}	6.53±0.21^{ab}	**10.90±0.27^{b}**
	Seri 0.50%	2.80±0.12^{ab}	0.97±0.12^{a}	7.13±0.24^{ab}	**10.90±0.27^{a}**	2.90±0.10^{a}	1.10±0.06^{a}	6.23±0.21^{a}	**10.23±0.23^{a}**
	Seri 1.00%	3.13±0.14^{c}	1.33±0.12^{c}	7.73±0.26^{bc}	**12.20±0.33^{b}**	3.27±0.13^{bc}	1.37±0.09^{b}	6.90±0.24^{b}	**11.53±0.30^{b}**
	Average	**2.95±0.05^{qy}**	**1.17±0.05^{y}**	**7.43±0.11^{ry}**	**11.55±0.14^{y}**	**3.20±0.05^{qy}**	**1.27±0.04^{py}**	**6.79±0.10^{ry}**	**11.27±0.13^{y}**

Means bearing different superscripts between sericin levels (abc) and between segmental defects (pqr) at each stage, and between stages (xy) differ significantly (p<0.05)

No report could be seen in the literature on sperm morphological studies employing different levels of Sericin to compare our findings of beneficial effect of 0.5 or 0.25 % Sericin. However, the present findings of total sperm abnormalities were in harmony with the previous reports of Patel *et al.* (2016), Rao *et al.* (2017) and Demra *et al.* (2017) using different additives, but the levels of sperm abnormalities reported by them were higher than our findings in either of species. Mandal *et al.* (2009) reported abnormalities of sperm head, mid piece, tail and total in frozen-thawed semen of Sahiwal bulls as 3.92±0.44, 8.27±0.73, 7.18±0.65 and 19.37±0.95 per cent, respectively, which are quite higher than the present findings in Gir and Murrah bull semen. However, Chaudhari *et al* (2015) and Chaudhary *et al.* (2018) documented sperm head, mid piece, tail and total abnormalities in the pre-freeze and post-thaw semen of Surti and Gir bulls to be significantly lower in Optixcell than Bioxcell, Andromed or even standard TFYG extender, and their values were very close to the present findings in both the species. Similarly, Varghese *et al.* (2015) observed beneficial effect of cysteine @ 1 mg/ml and taurine 4 mg/ml supplementation in TFYG extender on overall freezability including segmental and total sperm abnormalities of Surti buffalo semen with values in accordance to the present findings. Chikhaliya *et al.* (2018) demonstrated significant beneficial effect on sperm abnormalities of Gir bull semen at both pre-freeze and post-thaw stage in Andromed with 50 mM taurine as compared to higher or lower levels and control dilutor, but their values of total sperm abnormalities were almost double than the present findings.

Shaikh *et al.* (2016) reported significantly higher sperm motility (64.16±0.52%) and viability (64.16±0.52 %) and lower total sperm abnormalities (5.00±0.21 %) in Kankrej bull semen frozen in TFYG dilutor with trehalose at 100 mM than at 50 or 150 mM and control dilutor. Rao *et al.* (2017) reported supplementation of vitamin E @ 1.0 % or Vit-C at 5 mM and in combination in TFYG to be significantly ($p<0.05$) beneficial in improving post-thaw sperm motility (50.69±0.02 %) and viability (56.01±0.01 %) with reduced sperm abnormalities (16.02±0.02 %) in crossbred bull semen as compared to higher or lower levels and control diluent, although their values of all the traits were higher than our findings at post-thaw stage in both the breeds.

Sperm Acrosomal and Plasma Membrane Integrity

The mean percentages of spermatozoa with intact acrosomes observed initially on dilution and at post-thaw stage in semen of Gir bulls, irrespective of Sericin levels, were 89.86±0.20 and 82.58±0.23, and in Murrah bulls 92.30±0.19 and 85.13±0.18, respectively. Similarly, the mean percentages of HOS reactive spermatozoa recorded initially and at post-thaw stage in semen of Gir bulls were 71.19±0.45 and 39.56±0.57, and in Murrah bulls

71.62±0.42 and 39.33±0.54, respectively. The values of both the traits differed highly significantly ($p<0.01$) between stages, but not between breeds (Table 2). The initial and post-thaw intact acrosome and HOS reactive sperm per cent were significantly ($p<0.05$) higher for both the breeds in TFYG with 0.50 and/or 0.25 % sericin as compared to higher or lower levels and control tris extender, which were however statistically similar. Further, the bull effect was significant for HOS reactive sperm per cent at both the stages in both the breeds. Thus, the supplementation of TFYG extender with Sericin 0.5 and/or 0.25 % gave good results with respect to all the parameters due to its cryoprotectant and antioxidant effect on account of higher amount of hydroxyl groups of hydroxyamino acids (serine and threonine) (Kwang *et al.* 2003), and higher level, *i.e.,* 1.0 % was observed to be detrimental to some of the sperm quality parameters. The sperm acrosomal and plasma membrane integrity has direct relationship with fertilizing potential of given spermatozoa. The present findings on acrosome integrity were in harmony with the previous report of Rana *et al* (2003) and Demra *et al* (2017), but Beheshti *et al* (2013) and Rao *et al* (2017) revealed lower values than our findings. Demra *et al* (2017) found significantly ($p<0.05$) higher post-thaw acrosome integrity (87.2±1.6 %) and HOS reactivity (20.7±0.9%) of buffalo sperm cryopreserved in TFYG extender with Sericin @ 0.25 % as compared to higher levels and control dilutor, while Kumar *et al*. (2015) and Dorji *et al.* (2015) reported significantly higher HOS reactive sperm per cent in post-thawed bull semen with Sericin @ 0.5 % level.

Sariozkan *et al*. (2009) reported significantly higher acrosome integrity in TFYG with cysteine 2 mM or taurine 2 mM compared to control extender, but no significant effect was observed on HOS reactive sperm (48.4±1.9) during the freeze-thawing process. Varghese *et al.* (2015) observed significant beneficial effect of cysteine 1 mg/ml and taurine 4 mg/ml in TFYG extender over lower levels or control extender on acrosome as well as plasma membrane integrity of Surti buffalo semen before and after cryopreservation. Similar were the observations of Chikhaliya *et al.* (2018) for Gir bull semen frozen in Andromed extender supplemented with taurine at 50 mM as compared to 25 or 75 mM and control dilutor. Rao *et al.* (2017) documented significantly ($p<0.05$) higher post-thaw acrosome integrity (63.57 ±0.01 %) and HOS reactivity (45.83±0.01 %) of crossbred bull semen in TFYG extender supplemented with vitamin-E @ 1 mg/ml as compared to control. Shaikh *et al* (2016) reported significantly better acrosome integrity (80.91±0.43) and HOS reactivity (73.91±0.35) of Kankrej bull semen in TFYG with trehalose at 100 mM as compared to 50- or 150-mM level and control extender. Moreover, the acrosome integrity was in accordance, but HOST value was much higher than our findings in either of the breeds.

Post-Thaw Incubation Survival of Bovine Sperm

The post-thaw survival of sperms was significantly ($p<0.05$) better in TFYG extender with 0.5% Sericin, followed by TFYG with 0.25% and 0.1% Sericin at all incubation intervals in semen of both Gir and Murrah bulls (Table 1). The results displayed that all levels of sericin could sustain tolerable level of sperm survival at least for 1 hr after thawing. Supplementation of Sericin at 1 % level had no any advantage over non-added control extender, and in fact it deteriorated motility at certain instances. Therefore, frozen semen once thawed should be utilized as early as possible within 1 hr for AI to obtain better conception rates. These findings on post-thaw longevity of cryopreserved bovine spermatozoa are in harmony with the previous reports of Dhami *et al.* (1994), Varghese *et al.* (2015), Chaudhari *et al.* (2015) and Chaudhary *et al.* (2018) on Gir, Murrah and Surti bull semen. However, Taraphder *et al.* (2001), Rana *et al.* (2003) and Chowdhary *et al.* (2013) revealed relatively higher values than our findings at different incubation intervals. Rastegarnia *et al.* (2013) reported higher post-thaw motility and viability of semen till 4 hrs of incubation in buffalo bull semen.

Many early workers have shown that the frozen spermatozoa survived for a much shorter time following thawing than spermatozoa that were not frozen, since they were extensively damaged under ideal freezing-thawing conditions or were adversely affected by temperature fluctuation or type of extender (Brown *et al.,*1982, Rastegarnia *et al.,* 2013). Kedia *et al.* (2013) indicated that motility may remain good, but increasing post-thaw incubation duration affects cell membrane permeability and cervical mucus penetration ability of spermatozoa, which may affect fertility. However, no report could be seen in the literature depicting post-thaw incubation study of bovine semen cryopreserved in presence of Sericin, although use of other antioxidants such as cysteine, taurine in TFYG extender (Dhami *et al.,*1994; Varghese *et al.,* 2015), and soybean based extenders (Chaudhari *et al.,* 2017, Chaudhary *et al.,* 2018) have been reported to prolong the post-thaw survival of bovine spermatozoa with better progressive motility in comparison to control conventional extenders.

Conclusion

It is concluded that significantly higher sperm progressive motility, better viability with acrosomal and plasma membrane integrity, and fewer sperm abnormalities observed at all stages of cryopreservation including better post-thaw longevity of bull and buffalo semen extended with TFYG extender having Sericin 0.5 and/or 0.25 % over 0.1 or 1.0 % level and control TFYG extender, may be due to its antioxidant and cryoprotective properties by reducing ROS production and scavenging the free radicals generated during dilution, cryopreservation and thawing of bovine semen. Supplementation of TFYG with Sericin 0.5 and/or 0.25 % gave good results with respect to all the parameters on account of its higher amount of hydroxyl groups of hydroxy-amino acids (serine and threonine), however higher level (1.0 %) was observed to be detrimental to some of the sperm quality parameters.

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Conflict of Interests

There is no conflict of interest.

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References

1. Beheshti, R., Yosefi, M., Eshratkhah, B. and Ghalekandi, J.G. (2013). Effects of addition of taurine to semen extenders on microscopic factors of buffalo bulls sperm after thawing. *Buffalo Bulletin*, 32, 49-53.
2. Brown, J.L., Senger, P.L. and Hiller, J.K. (1982). Influence of thawing time and post-thaw temperature on ac rosomal maintenance and motility of bovine spermatozoa frozen in 0.5 ml French straws. *Journal of Animal Science,* 54(5), 138-144.
3. Celeghini, E.C.C., Arruda, R.P., Andrade, A.F.C., Nascimento, J., Raphael, C.F. and Rodrigues, P.H. M. (2008). Effects that bovine sperm cryopreservation using two different extenders has on sperm membranes and chromatin. *Animal Reproduction Science,* 104, 119-131.
4. Chhillar, S., Singh, V.K., Kumar, R. and Atreja, S.K. (2012). Effects of taurine or trehalose supplementation on functional competence of cryopreserved Karan Fries semen. *Animal Reproduction Science,* 135(1-4), 1-7.
5. Chikhaliya, P.S., Borakhatariya, D.N., Solanki, G.B. and Ahlavat A.A. (2018). Effect of addition of taurine in Andromed extender for cryopreservation of Gir bull semen. *Proceeding of the 34th Annual Convention of ISSAR and International Symposium* held at Veterinary College, AAU, Anand, December 28-30, p.
6. Chaudhari, D.V., Dhami, A.J., Hadiya, K.K. and Patel, J.A. (2015). Relative efficacy of egg yolk and soya milk-based extenders for cryopreservation (−196° C) of buffalo semen. *Veterinary World*, 8(2), 239-244.
7. Chaudhary, P.J., Dhami, A.J., Chaudhari, D.V., Hadiya, K.K. and Patel J.A. (2018). Efficacy of egg yolk based and egg yolk free soya bean milk-based extenders for cryopreservation of Zebu cattle and buffalo semen. *Indian Journal of Animal Research,* 52(8), 1134-1140
8. Chowdhury, S., Das, S., Gupta, T., Sana, D. and Bose, S. (2013). A comparative study with Murrah buffalo and indigenous Gir spermatozoa to hypo-osmotic swelling test. *Exploratory Animal Medical Research*, 3(2), 159-164.
9. Cotran, R.S., Kumar, V. and Robins, S.L. (1989). *Pathologic Basis of Diseases*, 4th Edn, W.B. Saunders Co., Philadelphia, pp. 9-16.
10. Demra, R., Gandotra, V.K., Singh, A.K. and Kumar, A. (2017). Validation of Sericin concentration in semen freezing media and its effect on post-thaw quality of buffalo bull semen. *Veterinary Practitioner*, 18(1), 42-44.
11. Dhami, A.J., Jani, V.R., Mohan, G. and Sahni, K.L. (1994). Effect of extenders and additives on freezability, post-thaw thermo resistance and fertility of frozen Murrah buffalo semen under tropical climate. *Buffalo Journal,* 1, 35-45.
12. Dorji, P., Vongprolub, T., Pattarajinda, V., Chankitisakul, V. and Chuawongboon, P. (2015). Effect of sericin

supplement in Tris-egg yolk citrate extender on quality of cryopreserved semen from Thai native bulls. *Bhutan Journal of Natural Resources & Devevelopment*, 2(1), 11-18.
13. Kadirvel, G., Kumar, S. and Kumaresan, A. (2009). Lipid peroxidation, mitochondrial membrane potential and DNA integrity of spermatozoa in relation to intracellular reactive oxygen species in liquid and frozen-thawed buffalo semen. *Animal Reproduction Science,* 114(1-3), 125-134.
14. Kedia, N.K., Tiwari, R.P., Mishra, G.K., Awasthi, M.K. and Sahu, S.B. (2013). Effect of post- thaw incubation on quality of frozen Tharparkar bull semen. *Indian Journal of Animal Reproduction,* 34, 9-12.
15. Kumar, P., Kumar, D., Sikka, P. and Singh, P. (2015). Sericin supplementation improves semen freezability of buffalo bulls by minimizing oxidative stress during cryopreservation. *Animal Reproduction Sciene,* 152, 26-31.
16. Kumar, R., Mohanarao, G.J. and Atreja, S.K. (2011). Freeze-thaw induced genotoxicity in buffalo (*Bubalus bubalis*) spermatozoa in relation to total antioxidant status. *Molecular Biology Reports*, 38(3), 1499-1506.
17. Kurmi, D.J., Sinha, M.P., Kumar, R., Hazarika, S.B., Dewry, R.K. and Gohain, S. (2018). Effect of vitamin E on the quality of frozen ram semen. *Theriogenology Insight*, 8(1), 39-43.
18. Kwang, Y.C., Jae, Y.M., Yong, W.L., Kwang, G.L., Joo, H.Y., Hae, Y.K., Ki, H.K. and Chong, S.C. (2003). Preparation of self-assembled silk sericin nanoparticles. *International Journal of Biology and Macromolecules*, 32,36-42.
19. Mondal, D.K., Tyagi, S. and Kumar, M. (2009). Assessment of sperm morphological abnormalities, liability and plasmallemal integrity in frozen-thawed semen of Sahiwal bulls. *Indian Journal of Animal Sc*iences, 79(4), 358-361.
20. Patel, H.A., Siddiquee, G.M., Chaudhari, D.V. and Suthar, V.S. (2016). Effect of different antioxidant additives in semen diluent on cryopreservability (−196°C) of buffalo semen. *Veterinary World*, 9(3), 299-303.
21. Patel, T.M., Dhami, A.J., Chaudhari, D.V. and Pathan, M.M. (2019). Influence of antioxidant sericin in tris extender on oxidative markers during cryopreservation (-196ºC) of bovine semen. *The Indian Journal of Veterinary Science & Biotechnology*, 15(2), 34-37.
22. Rana, C.M., Dhami, A.J., Zalu, G.K., Vibhakar, K.V. and Landani, N.G. (2003). Preservation of Gir (*Bos indicus*) and Jafarabadi (*Bubalus bubalis*) semen at 5°C and ultralow temperature using Sephadex filtration technique. *Indian Journal of Animal Sciences*, 73(8), 858-863.
23. Rao, T.K.S., Mohanty, T.K. and Bhakat, M. (2017). Assessment of antioxidants for preservation of crossbred bull semen in Tris based extender. *Indian Journal of Animal Research.*, 51(6), 993-997.
24. Rastegarnia, A., Shahverdi, A., Rezaei, T. and Shafiepour, V. (2013). *In vitro* comparison of soybean lecithin-based extenders for cryopreservation of buffalo (*Bubalus bubalis*) semen. *Comparative Clinical Pathology*, 22(1), 1-10.
25. Rasul, Z., Ahmad, N. and Anzar, M. (2001). Changes in motion characteristics, plasma membrane integrity, and acrosome morphology during cryopreservation of buffalo spermatozoa. *Journal of Andrology*, 22, 278-283.
26. Reddy, N.S.S., Mohanarao, G.J. and Atreja, S.K. (2010). Effects of adding taurine and trehalose to a tris-based egg yolk extender on buffalo (*Bubalus bubalis*) sperm quality following cryopreservation. *Animal Reproduction Science,* 119(3-4), 183-190.
27. Sarıözkan, S., Bucak, M.N., Tuncer, P.B., Ulutaş, P.A. and Bilgen, A. (2009). The influence of cysteine and taurine on microscopic–oxidative stress parameters and fertilizing ability of bull semen following cryopreservation. *Cryobiology*, 58(2), 134-138.
28. Shaikh, K.Q., Nakhashi, H.C., Suthar, B.N., Sutaria, P.T. and Sharma, V.K. (2016). Comparison of fresh semen parameters with frozen thawed semen following incorporation of trehalose. *Life Sciences Leaflets*, 76, 107-115.
29. Taraphder, S., Gupta, A.K., Raina, V.S. and Tomar, S.S. (2001). Effect of incubation (37°C) on post-thaw motility of Murrah buffalo bull spermatozoa. *Indian Journal of Dairy Science*, 54(2), 80-83.
30. Varghese Orin, Dhami, A.J., Hadiya, K.K., Patel, J.A. and Parmar, S.C. (2015). Role of antioxidants cysteine and taurine in tris egg yolk-based extender for cryopreservation of Surti buffalo semen. *Indian Journal of Animal Reproduction,* 36(2), 39-45.
31. Yasmin, C., Otoi, T., Setiadi, M. and Karja, N. (2015). Maturation and fertilization of sheep oocytes cultured in serum-free medium containing silk protein sericin. *Acta Veterinarcia Hungarica*, 63(1), 110-117.

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