International Journal of Livestock Research, Vol. 10 (12) Dec' 2020
http://www.ijlr.org
eISSN: 2277-1964
NAAS Rating 2020 – 5.36

Original Research

Effect of Mifepristone, Sericin and Taurine in Tris Extender on Oxidative Markers and Quality of Fresh and Frozen-Thawed Bovine Spermatozoa

Devangana Chaturvedi[1], A. J. Dhami[1]*, D. V. Chaudhari[1] and M. M. Pathan[2]

[1]Department of Veterinary Gynaecology & Obstetrics, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand, Gujarat, INDIA
[2]Department of Physiology & Biochemistry, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand, Gujarat, INDIA

*Corresponding Author: ajdhami@aau.in

How to cite this paper: Chaturvedi, D., Dhami, A. J., Chaudhari, D. V., & Pathan, M. M. (2020). Effect of Mifepristone, Sericin and Taurine in Tris Extender on Oxidative Markers and Quality of Fresh and Frozen-Thawed Bovine Spermatozoa. *International Journal of Livestock Research*, 10(12), 61-67. doi: http://dx.doi.org/10.5455/ijlr.20200930010456

Received : Sep 30, 2020
Accepted : Oct 28, 2020
Published : Dec 31, 2020

Abstract

This study was carried out on semen of 3 Gir and 3 Murrah bulls. Semen ejaculates with >75 % initial motility (n=7 per bull; 21/breed; total 42) were split-diluted at 100 million sperm per ml using Tris-citric acid-fructose-egg yolk-glycerol (TFYG) extender without and with Mifepristone (10 µg/ml), Sericin (5 mg/ml) and Taurine (4 mg/ml) as additives. French mini straws were filled and sealed using IS4 system, and frozen in liquid nitrogen vapour using a programmable biofreezer after 4 hrs of equilibration. The straws were thawed in water bath at 37 ℃ for 30 sec. The overall mean per cent sperm motility observed in freshly diluted and frozen-thawed semen, irrespective of antioxidants, were 83.57±0.51 and 46.73±0.67 in Gir, and 83.15±0.54 and 48.81±0.82 in Murrah bulls, respectively. The corresponding per cent capacitated sperm (B+AR pattern) in chlortetracycline (CTC) fluorescence assay were 17.77±0.53 and 34.44±0.95 in Gir, and 18.00±0.51 and 31.20±0.82 in Murrah bulls. The mean seminal plasma MDA (µmol/ml) activity on dilution and at post-thaw stage were 18.83±0.25 and 20.59±0.28 for Gir, and 19.32±0.26 and 21.76±0.31 for Murrah bulls, respectively. The corresponding means for SOD (U/ml) were 2.94±0.05 and 2.45±0.03 for Gir, and 2.35±0.06 and 1.97±0.05 for Murrah bulls, and the GPx (nmol/min/ml) 493.85±8.31 and 438.87 ±8.13 for Gir, and 508.82±8.71 and 452.83±8.20 for Murrah bulls, respectively. All the parameters differed significantly between stages and between extender-additives. MDA activity and capacitated sperm were significantly ($p<0.05$) lower, while SOD and GPx were higher with improved sperm motility ($p<0.01$), particularly at post-thaw stage, in extender fortified with Mifepristone than the control, but statistically similar to extender fortified with Sericin and Taurine. In conclusion, Mifepristone 10 µg/ml in TFYG extender appeared to be the best in terms of antioxidant and capacitation inhibitor followed by Taurine 4 mg/ml and Sericin 5 mg/ml over control extender against ROS mediated injuries during cryopreservation of both cattle and buffalo semen as assessed by sperm motility, CTC assay and oxidative makers.

Keywords: Antioxidant, Mifepristone (RU-486), Bovine Semen, Cryocapacitation, CTC Assay, Oxidative Markers

Introduction

The reactive oxygen species (ROS) mediated oxidative stress is known to deteriorate the quality of frozen semen (Baumber *et al.,* 2005), which subsequently increases lipid peroxidation in the spermatozoa (El-Sisy *et al.,* 2007; Kadirvel *et al.,* 2009) affecting its plasma membrane and DNA integrity and thereby reducing its potential fertility. Different types of antioxidants both enzymatic and non-enzymatic have been fortified into the semen extenders with varying degree of success by many workers (Orin *et al.,* 2015; Chikhaliya *et al.,* 2018; Patel *et al.,* 2019), indicating their crucial role in improving the preservability and fertility of the bull and buffalo semen. The antioxidative systems control the balance between production and neutralization of ROS and protect spermatozoa against peroxidative damage.

Antioxidant potential of silk protein sericin was reported by Kato *et al.* (1998), as suppressed lipid peroxidation and inhibited tyrosinase activity *in vitro* by its scavenging function mediated through chelating effect of hydroxyl groups of hydroxyamino acids (serine and threonine) that are abundantly contained in sericin (Kwang *et al.,* 2003). Sericin also possesses the biological activity of preventing cell death during culture and cryopreservation (Masakazu *et al.,* 2003), and it has been proved beneficial in sperm cryopreservation (Kumar *et al.,* 2015; Dorji *et al.,* 2016; Demra *et al.,* 2017). Taurine, a sulphonic amino acid and permeating cryoprotectant acts as an antioxidant and causes membrane lipid and protein rearrangement, which results in increased membrane fluidity, greater dehydration at lower temperatures, and therefore increased ability of spermatozoa to survive during cryopreservation (Holt, 2000, Chhillar *et al.,* 2012). Mifepristone (RU 486) with steroid structure, an antiprogestin compound, @ 10 µM protected the sperm from premature capacitation as measured by intracellular calcium level, expression of tyrosine phosphorylated proteins, antioxidant status and CTC assay of buffalo spermatozoa (Dalal *et al.,* 2019).

Literature on comparative antioxidant and capacitation inhibitory potential of Mifepristone, Sericin and Taurine is meagre particular on buffalo sperm. Hence, the present study was conducted to evaluate the comparative antioxidant and capacitation inhibitory properties of Mifepristone, Sericin and Taurine through assay of sperm motility, capacitation status and oxidative markers before and after freezing of cattle and buffalo semen in Tris extender.

Materials and Methods

The present study was carried out on semen of three healthy mature breeding bulls each of Gir and Murrah breed, aged between 6 and 9 years, at the College of Veterinary Science, AAU, Anand, Gujarat (India) during September to March 2019-20. All the bulls were in good health, dewormed and were vaccinated against common contagious diseases. They were maintained in nearly identical nutritional and managerial conditions with twice a week semen collection schedule. Semen was collected using artificial vagina from each bull in the morning hours over a dummy buffalo bull. Three antioxidant cryocapacitation inhibitory additives at the levels found optimum based on previous studies at our station and/or available literature were used, *viz.,* Mifepristone (10 µg/ml, RU-486$^{@}$, Sigma-Aldrich, USA, Dalal *et al.,* 2019), Sericin (5 mg/ml, Sigma-Aldrich, USA, Patel *et al.,* 2019) and Taurine (2 amino-methane sulphonic acid @ 4 mg/ml, Central Drug House Ltd., New Delhi, Orin *et al.,* 2015).

Semen ejaculates (n=7 per bull; 21 per breed) with >75% initial motility were split into four aliquots and were extended at 34ºC @ 100 million sperm per ml with Tris-citric acid-fructose-egg yolk-glycerol (TFYG) extender without and with selected additives, *viz.,* Mifepristone (10 µg/ml), Sericin (5 mg/ml) and Taurine (4 mg/ml). The extended aliquots were soon filled and sealed in French mini straws by IS4 machine, cooled to 5ºC, equilibrated for 4 hrs and frozen in liquid nitrogen vapour using a bio-freezer (IMV, France) employing standard freezing protocol for bovine semen. The straws were thawed in water bath at 37°C for 30 sec. The freshly diluted as well as frozen-thawed samples of each ejaculate were assessed subjectively for the individual sperm motility, CTC (chlortetracycline) fluorescence assay for A, B and AR pattern (Dalal *et al.,* 2019) and seminal plasma oxidative enzymes (MDA, SOD and GPx) status. For seminal plasma separation, 2.0 ml each of freshly diluted and frozen-thawed semen samples of different aliquots were centrifuged at 1000 g for 10 minutes, and the supernatants collected were stored at -20° C in deep freeze. The determination of activities of antioxidant enzymes, *viz.,* glutathione peroxidase (GPx), superoxide dismutase (SOD) and malondialdehyde (MDA) in pre- and post-thaw plasma was carried out using commercial kits (Cayman Assay Kits, USA, Cat No. 706002, 705003 & 703102) according to the instructions of manufacturer. The data was analyzed statistically using completely randomized design and Duncan's multiple range test within the breed to know the effect of additives, and 't' test to know breed difference in each trait. The percentage values were arcsin transformed before analysis (Snedecor and Cochran, 1994).

Results and Discussion

The mean values of sperm motility, capacitated sperm and anti-oxidative enzymes, *viz.*, MDA, SOD and GPX recorded in freshly diluted and frozen-thawed bovine semen extended with control TFYG extender and TFYG fortified with Mifepristone, Sericin and Taurine are presented in Table 1 and 2.

Motility and Capacitation of Sperm

The mean percentages of sperm motility observed in freshly diluted and frozen-thawed semen, irrespective of antioxidants, were 83.57±0.51 and 46.73±0.67 in Gir, and 83.15±0.54 and 48.81±0.82 in Murrah bulls, respectively. The corresponding per cent capacitated sperm (B + AR pattern) recorded were 17.77±0.53 and 34.44±0.95 in Gir, and 18.00±0.51 and 31.20±0.82 in Murrah bull semen. The mean percentages of progressively motile and capacitated (B+AR pattern) sperm differed significantly ($p<0.01$) between extender-additives with better results in extender fortified with Mifepristone followed by Taurine and Sericin as compared to Control TFYG extender both at pre-freeze and post-thaw stage in Gir and Murrah bull semen. The average post-thaw values of these parameters were significantly ($p<0.01$) better in buffalo semen as compared to cattle semen. The bull and bull x additive interaction effects were statistically non-significant before and after freezing of semen in both the breeds. The values in presence of Sericin and Taurine were statistically similar in both the breeds, and significantly better than the control extender, yet inferior than the extender fortified with Mifepristone, particularly at post-thaw stage (Table 1).

Table 1: Effect of Mefepristone (RU-486), sericin and taurine in Tris extender on progressive sperm motility and capacitation status (B+AR pattern) of Gir and Murrah bulls spermatozoa during cryopreservation

Freezing stage	Extender-Additives	Sperm progressive motility (%)		Capacitated sperm (%)	
		Gir	Murrah	Gir	Murrah
On dilution	Control	77.14±1.41^{a}	76.67±1.24^{a}	23.71±1.24^{a}	23.19±1.20^{a}
	RU-486	87.14±1.13^{c}	86.90±1.26^{c}	12.90±0.93^{c}	23.29±0.99^{c}
	Sericin	85.09±0.61^{b}	84.52±1.02^{b}	17.29±1.10^{b}	17.57±1.31^{b}
	Taurine	85.10±0.63^{b}	84.52±1.18^{b}	17.19±1.24^{b}	17.95±1.23^{b}
	Average	**83.57±0.51**	**83.15±0.54**	**17.77±0.53**	**18.00±0.51**
Post-thaw	Control	41.90±1.93^{a}	44.05±2.78^{a}	44.66±1.83^{a}	40.24±1.76^{a}
	RU-486	51.19±1.88^{c}	53.81±2.15^{c}	26.00±1.95^{c}	23.48±1.59^{c}
	Sericin	47.14±2.03^{b}	48.33±2.63^{ab}	33.52±2.44^{b}	30.86±1.80^{b}
	Taurine	46.67±2.18^{b}	49.05±2.65^{b}	33.57±2.25^{b}	30.24±1.90^{b}
	Average	**46.73±0.67***	**48.81±0.82**	**34.44±0.95****	**31.20±0.82**

*Means bearing different superscripts between additives (abc) at each stage differ significantly ($p<0.05$) for a breed. *$p<0.05$, **$p<0.01$ between breed.*

The present findings in control TFYG extender during cryopreservation concurred well with the previous reports of Maurya *et al.* (2003), Chaudhary *et al.* (2018) and Patel *et al.* (2020) either in cattle or buffalo semen cryopreserved with same extender. Significant improvement in post-thaw sperm quality with respect to motility, viability, CTC assay and oxidative markers of Murrah buffalo semen cryopreserved with inclusion of Mifepristone (RU 486) at the dose rate of 10 µM in tris extender as compared to higher or lower levels and control extender has been reported by Dalal *et al.* (2019), which concurred well with the present findings in both the breeds/species. Similarly, Reddy *et al.* (2010), Chhillar *et al.* (2012), Orin *et al.* (2015) and Chikhaliya *et al.* (2018) reported significant ($p<0.05$) increase in pre-freeze and/or post-thaw motility of bovine sperm in TFYG extender supplemented with taurine or trehalose (50-100 mM). Kumar *et al.* (2015), Dorji *et al.* (2016) and Patel *et al.* (2020) found significantly ($p<0.05$) higher pre-freeze and/or post-thaw motile, live and HOS reactive sperm in cattle and buffalo semen cryopreserved in TFYG extender with Sericin @ 0.50 % as compared to higher or lower levels and control extender, while Demra *et al*. (2017) found non-significantly higher ($p>0.05$) post-thaw values with 0.25 % sericin as compared to control TFYG extender and higher levels of sericin.

Our findings of CTC assay in fresh and post-thaw semen are in accordance with previous reports of Cormier *et al.* (1997), Bailey *et al.* (2003), Chhillar *et al.* (2012), Zodinsanga *et al*. (2015) and Longobardi *et al.* (2017^{a,b}) for bull semen. The results indicated that the cryopreservation process induces precocious capacitation of bull sperm restricting *in vivo* fertile lifespan of frozen-thawed sperm. Dalal *et al.* (2019) confirmed that 10 µM Mifepristone

protects the sperm from premature capacitation measured by intracellular calcium level, expression of tyrosine phosphorylated proteins and CTC assay of buffalo spermatozoa, and similar were the observations of Chhillar *et al.* (2012) for Taurine.

Lipid Peroxidation / Malondialdehyde Production

The mean seminal plasma Malondialdehyde (MDA) activity on dilution and at post-thaw stage, irrespective of additives, for Gir bulls were 18.83±0.25 and 20.59±0.28 µmol/ml, and for Murrah bulls 19.32±0.26 and 21.76±0.31 µmol/ml, respectively, which differed significantly ($p<0.01$) between breeds at post-thaw stage, being higher in buffalo semen. The mean values of MDA activity in seminal plasma differed significantly ($p<0.05$) between extender-additives only at post-thaw stage. Seminal plasma MDA activity was significantly lower in extender supplemented with Mifepristone than the control, and it was statistically similar in extender supplemented with Sericin and Taurine. The MDA activity in letter two additives was lower yet statistically similar to control extender. The present findings on lipid peroxidation concurred well with the earlier reports of Asadpour *et al.* (2012), Chikhaliya *et al.* (2018) and Patel *et al.* (2019), who also found higher values of MDA in Zebu bulls than the present findings in Gir bulls.

Shaikh *et al.* (2016) recorded significantly ($p<0.05$) lower lipid peroxidation (MDA, 20.06±0.13 µmol/ml) in Kankrej bull semen cryopreserved in TFYG extender supplemented with trehalose at 100 mM compared to 50 or 150 mM and control extender, and their values compared well with the present findings. Kumar *et al.* (2015) and Dorji *et al.* (2016) also reported lower levels of MDA production in cryopreserved Murrah and Thai bull semen with Sericin @ 0.50 % (w/v) in TFYG extender as compared to control extender. Likewise, Dalal *et al.* (2019) and Patel *et al.* (2019) reported significantly lower oxidative stress on sperm cryopreserved in TFYG extender supplemented with 10 µM Mifepristone and 0.5 % Sericin, respectively, as compared to higher or lower levels and control extender in terms of MDA, SOD and GPx activity of bull and buffalo spermatozoa, however their values were somewhat higher than the present ones. In an earlier study, significantly ($p<0.05$) lower rate of H_2O_2 production, lipid peroxidation and intracellular calcium in bull spermatozoa cryopreserved in TFYG and Andromed extenders fortified with 50 mM taurine and 100 mM trehalose as compared to control extender has been documented (Chhillar *et al.* 2012; Chikhaliya *et al.,* 2018), and similar were the results with 2 mM cysteine, but 2 mM taurine significantly increased MDA production in cryopreserved bovine semen (Sariozkan *et al.*, 2009).

Superoxide Dismutase and Glutathione Peroxidase Activity

The mean values of seminal plasma Superoxide Dismutase (SOD) activity on dilution and at post-thaw stage in TFYG extender, irrespective of additives, were 2.94±0.05 and 2.45±0.03 U/ml for Gir bulls, and 2.35±0.06 and 1.97±0.05 U/ml for Murrah bulls, respectively, which differed significantly ($p<0.01$) between breeds at both the stages, being lower in Murrahs. The corresponding values of mean seminal plasma Glutathione Peroxidase (GPx) activity were 493.85±8.31 and 438.87±8.13 nmol/min/ml for Gir, and 508.82±8.71 and 452.83±8.20 nmol/min/ml for Murrah bulls, respectively, without breed difference at any stage. The mean values of SOD and GPx activity in seminal plasma of Gir and Murrah bulls differed significantly ($p<0.05$) between extender-additives at both the stages. SOD activity was significantly ($p<0.05$) higher in extender fortified with Mifepristone than the control extender, but was statistically at par with extender supplemented with Taurine and Sericin in both the species, at both the stages. Seminal plasma GPx activity was significantly lower in control TFYG extender than the extender supplemented with Mifepristone, however the values with Sericin and Taurine were intermediary in both the breeds at both the stages. Further, the bull variation was significant ($p<0.05$) for both these enzymes on dilution and at post-thaw stage in both the breeds.

The SOD and GPx values recorded in TFYG extender without and with different antioxidants followed the trend of motile sperm during the steps of cryopreservation of bull and buffalo semen. The present findings of SOD activity are, however, comparatively lower than those of Kaka *et al.* (2016). Sariozkan *et al.* (2009) observed significantly ($p<0.001$) elevated SOD and GPx activity in TFYG extender with 2 mM cysteine as compared to control for cryopreserved bull semen. Kumar *et al.* (2015) and Patel *et al.* (2019) reported significantly ($p<0.05$) lower post-thaw MDA levels and higher SOD and GPx activity in bull and buffalo semen cryopreserved using TFYG extender with Sericin @ 0.50 % as compared to the control. These studies established protective role of Sericin against ROS mediated sperm cell cryoinjury. Shaikh *et al.* (2016) observed beneficial effect of trehalose 100 mM on oxidative stress in Kankrej bull semen frozen in TFYG extender. The lowest ($p<0.01$) level of MDA (4.46±0.31 nmol/ml)

production and higher GPx activity in goat semen cryopreserved in Tris with 75 mM taurine, and 50- and 75-mM trehalose compared to the control has also been reported by Atessahin *et al.* (2008).

Table 2: Effect of fortification of Tris extender with Mefepristone (RU-486), Sericin and Taurine on oxidative enzymes in seminal plasma of Gir and Murrah bulls before and after cryopreservation of semen

Freezing stage	Extender-Additives	Lipid Peroxidation (MDA, µmol//ml)		Superoxide Dismutase (SOD, U/ml)		Glutathione Peroxidase (GPx, nmol/min/ml)	
		Gir	Murrah	Gir	Murrah	Gir	Murrah
On dilution	Control	19.58±0.47	20.01±0.55	2.67±0.08^{a}	2.09±0.10^{a}	465.59±16.82^{a}	482.34±18.67^{a}
	RU-486	18.35±0.57	18.70±0.53	3.15±0.10^{b}	2.56±0.11^{b}	521.65±15.91^{b}	534.95±15.37^{b}
	Sericin	18.60±0.46	19.23±0.53	2.97±0.09^{b}	2.34±0.09^{ab}	489.84±16.02^{ab}	515.23±17.68^{ab}
	Taurine	18.78±0.52	19.33±0.49	2.96±0.08^{b}	2.42±0.13^{b}	498.33±16.51^{ab}	502.78±17.03^{ab}
	Average	**18.83±0.25**	**19.32±0.26**	**2.94±0.05****	**2.35±0.06**	**493.85±8.31**	**508.82±8.71**
Post-thaw	Control	21.50±0.54^{b}	22.88±0.63^{b}	2.29±0.06^{a}	1.77±0.08^{a}	414.17±16.64^{a}	427.30±16.17^{a}
	RU-486	19.42±0.63^{a}	20.90±0.57^{a}	2.57±0.07^{b}	2.11±0.10^{b}	463.16±14.48^{b}	483.75±14.28^{b}
	Sericin	20.59±0.50^{ab}	21.46±0.63^{ab}	2.41±0.06^{ab}	1.91±0.08^{ab}	438.49±16.14^{ab}	460.94±16.46^{ab}
	Taurine	20.34±0.54^{ab}	21.80±0.62^{ab}	2.51±0.07^{b}	2.09±0.12^{b}	439.66±17.08^{ab}	449.31±17.91^{ab}
	Average	**20.59±0.28****	**21.76±0.31**	**2.45±0.03****	**1.97±0.05**	**438.87±8.13**	**452.83±8.20**

Means bearing different superscripts between additives (abc) at each stage differ significantly ($p<0.05$) for a breed. $^{**}p<0.01$ between breed

According to Masakazu *et al.* (2003) sericin possesses the biological activity of preventing cell death during culture and cryopreservation, and therefore it is used in oocyte maturation media and culture media replacing bovine serum albumin and fetal bovine serum (Sasaki *et al.*, 2005; Aghaz *et al.*, 2016), and in sperm cryopreservation (Kumar *et al.*, 2015; Dorji *et al.*, 2016; Demra *et al.*, 2017; Patel *et al.*, 2019). The beneficial role of Sericin as cell protector, cryoprotector and antioxidant with greater ability to eliminate free radicals has been reported during the cryopreservation of different biological cells and tissues (Sasaki *et al.*, 2005). During present study, an increase in percentage of sperm motility and decreased capacitated sperm in Mifepristone, Sericin and Taurine supplemented semen showed their cryoprotective effects. Kato *et al.* (1998) and Kwang *et al.* (2003) explained antioxidant potential of silk protein sericin that it could suppress lipid peroxidation and inhibit tyrosinase (polyphenol oxidase) activity *in vitro* by its scavenging function through the chelating effect of hydroxyl groups of its hydroxyamino acids (serine and threonine). Similarly, the taurine, a sulfonated amino acid, found in the seminal plasma and oviductal fluid, is one of the major non-enzymatic scavengers that plays an important role in the protection of spermatozoa against ROS and lipid peroxidation (Saleh and Agarwal, 2002). This could be one of the reasons for improved post-thaw motility of frozen-thawed spermatozoa, diluted in presence of Taurine in the semen extender. The freezing media supplemented with Mifepristone, Sericin, Taurine thus probably reduce the harmful effect of lipid peroxidation thereby protecting calcium channel resulting in significantly greater post-thaw sperm quality of bovine semen.

Conclusion

The study conducted with fortification of Mifepristone 10 µg/ml, Sericin 5 mg/ml and Taurine 4 mg/ml in TFYG extender appeared effective in reducing cryodamage, cryo-capacitation and improved post-thaw sperm quality by ameliorating ROS mediated oxidative damage. Mifepristone supplementation in particular reduced the oxidative stress and enhanced inherent antioxidant enzyme activity (SOD, GPx) protecting lipid peroxidation (MDA) and cryocapacitation in both Gir cattle and Murrah buffalo semen. Further, the reduction in oxidative stress with, not only Mifepristone but, Sericin and Taurine supplementation also resulted in enhanced seminal parameters, *i.e.*, motility and capacitation status of sperm in both freshly extended and cryopreserved bovine semen, without breed or species specificity in their action. However, for final verdict to use it on a routine basis in commercial semen production needs to be supported through actual *in vivo* fertility trials.

Acknowledgement

We are grateful to the University authorities of AAU, Anand, and Dean, College of Veterinary Science and Animal Husbandry, Anand for the funds and facilities provided for this research work.

Conflict of Interests

There is no conflict of interest.

Publisher Disclaimer

IJLR remains neutral concerning jurisdictional claims in published institutional affiliation.

References

1. Aghaz, F., Hajarian, H. and Karami Shabankareh, H. (2016). *In vitro* culture medium (IVC) supplementation with sericin improves developmental competence of ovine zygotes. *Reproductive Biology*, 16(1), 87-90.
2. Asadpour, R., Jafari, R. and Tayefi-Nasrabadi, H. (2012). The effect of antioxidant supplementation in semen extenders on semen quality and lipid peroxidation of chilled bull spermatozoa. *Iranian Journal of Veterinary Research*, 13(3), 246-249.
3. Atessahin, A., Bucak, M.N., Tuncer, P.B. and Kızıl, M. (2008). Effects of anti-oxidant additives on microscopic and oxidative parameters of Angora goat semen following the freeze-thawing process. *Small Ruminant Research*, 77(1), 38-44.
4. Bailey, J., Morrier, A. and Cormier, N. (2003). Semen cryopreservation: Successes and persistent problems in farm species. *Canadian Journal of Animal Science*, 83, 393-401.
5. Baumber, J., Ball, B.A. and Linfor, J.J. (2005). Assessment of the cryopreservation of equine spermatozoa in the presence of enzyme scavengers and antioxidants. *American Journal of Veterinary Research*, 66(5), 772-779.
6. 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.
7. 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.
8. 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. *Proc. 34th Annual Convention of ISSAR and International Symposium* held at Veterinary College, AAU, Anand, India, December 28-30, p.
9. Cormier, N., Sirad, M.A. and Bailey, J. (1997). Premature capacitation of bovine spermatozoa is initiated by cryopreservation. *Journal of Andrology*, 18, 461-468.
10. Dalal, J., Kumar, P., Chandolia, R. K., Pawaria, S., Rajendran, R., Sheoran, S. and Kumar, D. (2019). A new role for RU486 (Mifepristone): It protects sperm from premature capacitation during cryopreservation in buffalo. *Scientific Reports*, 9(1), 1-10.
11. 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.
12. Dorji, P., Vongprolub, T., Pattarajinda, V., Chankitisakul, V. and Chuawongboon, P. (2016). Effect of sericin supplement in Tris-egg yolk citrate extender on quality of cryopreserved semen from Thai native bulls. *Bhutan Journal of Natural Resources & Development*, 2(1), 11-18.
13. El-Sisy, G.A., El-Nattat, W.S. and El-Sheshtawy, R.I. (2007). Buffalo semen quality, antioxidants and peroxidation during chilling and cryopreservation. *Journal of Veterinary Research*, 11, 55-61.
14. Holt, W.V. (2000). Fundamental aspects of sperm cryobiology: the importance of species and individual differences. *Theriogenology*, 53, 47-58.
15. 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.
16. Kaka, A., Haron, W., Leghari, R.A., Memon, M.I., Kaka, U., Mirani, A.H. and Kalwar, Q. (2016). Effect of *in-vitro* supplementation of polyunsaturated fatty acids on frozen-thawed bull sperm characteristics using Bioxcell® extender. *Journal of Pure & Applied Science*, 5(3), 399.
17. Kato, N., Sato, S., Yamanaka, A., Yamada, H., Fuwa, N. and Nomura, M. (1998). Silk protein, sericin, inhibits lipid peroxidation and tyrosinase activity. *Bioscience Biotechnology and Biochemistry*, 62(1), 145-147.
18. Kumar, P., Kumar, D., Sikka, P. and Singh, P. (2015). Sericin supplementation improves semen freezability of buffalo bull by minimizing oxidative stress during cryopreservation. *Animal Reproduction Science*, 152,

26-31.
19. 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 Biological Macromolecules*, 32, 36-42.
20. Longobardi, V., Albero, G., De Canditiis, C., Salzano, A., Natale, A., Balestrieri, A. and Gasparrini, B. (2017[a]). Cholesterol-loaded cyclodextrins prevent cryocapacitation damages in buffalo (*Bubalus bubalis*) cryopreserved sperm. *Theriogenology*, 89, 359-364.
21. Longobardi, V., Salzano, A., Campanile, G., Marrone, R., Palumbo, F., Vitiello, M. and Gasparrini, B. (2017[b]). Carnitine supplementation decreases capacitation-like changes of frozen-thawed buffalo spermatozoa. *Theriogenology*, 88, 236-243.
22. Maurya, V.P., Tuli, R.K., & Singh, P. (2003). Effect of filtration on freezability and acrosomal integrity of Murrah buffalo bull semen. *Indian Journal of Animal Sciences*, 73(9), 938-985
23. Masakazu, T., Kazuhisa, T., Hideyuki, Y., Hiroshi, T. and Shigeru, N. (2003). The silk protein, sericin, protects against cell death caused by acute serum deprivation in insect cell culture. *Biotechnology Letters*, 25, 1805-1809.
24. Orin Varghese, 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.
25. Patel, T.M., Dhami, A.J., Chaudhari, D.V. and Hadiya, K.K. (2020). Inclusion of sericin in tris egg yolk extender improves bovine sperm quality during cryopreservation (-196ºC). *International Journal of Livestock Research*, 10 (7): 118-125.
26. 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 Sciences & Biotechnology*, 15(2), 34-38.
27. Reddy, N., Mohanarao, G. 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, 183-190.
28. Saleh, A. and Agarwal, A. (2002). Oxidative stress and male infertility: from research bench to clinical practise. *Journal of Andrology*, 23, 737-752.
29. 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.
30. Sasaki, M., Kato, Y., Yamada, H. and Terada, S. (2005). Development of a novel serum-free freezing medium for mammalian cells using the silk protein sericin. *Biotechnology and Applied Biochemistry*, 42(2), 183-188.
31. 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.
32. Snedecor, G.W. and Cochran, W.G. (1994). *Statistical Methods*. 14th edn. Oxford and IBH Publishing House, New Delhi, India.
33. Zodinsanga, V., Mavi, P.S., Cheema, R.S., Kumar, A. and Gandotra, V.K. (2015). Relationship between routine analysis, sperm function and fertility tests of cattle bull semen. *Asian Journal of Animal Science*, 9(1), 37-44.

****