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Effect of semen extender supplementation with sericin on post-thaw dairy bull sperm quality and lipid peroxidation

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Abstract: Silk sericin plays a protective role in a variety of mammalian cells during cryopreservation. This study aimed to determine the effects of sericin supplementation to a freezing extender on sperm quality and lipid peroxidation in dairy bull sperm cryopreservation. Each semen sample of five Holstein-Friesian crossbred bulls was divided into four aliquots and diluted in a tris-egg yolk extender supplemented with different concentrations of sericin [0%, 0.25%, 0.5%, and 1.0% (wt/vol)]. Sperm motility (CASA), viability, acrosome integrity, mitochondrial membrane potential (fluorescent staining) and lipid peroxidation (malondialdehyde – MDA test) were analysed. The results show that the 0.25% and 0.5% sericin groups had the highest total sperm motility ($P < 0.05$). Sperm viability, acrosome integrity, and mitochondrial function were higher in the group supplemented with 0.25% sericin compared to the control and 1.0% ($P < 0.05$). Sericin supplementation with 0.25% and 0.5% significantly decreased MDA concentrations compared with the control ($P < 0.05$). In conclusion, supplementation of the semen freezing extender with sericin at the concentration of 0.25% significantly improved the post-thaw semen quality and reduced lipid peroxidation in Holstein-Friesian crossbred bulls.

Keywords: antioxidant; cattle; frozen semen; ROS; semen cryopreservation

Sperm freezing is an important biotechnological method for the transfer of superior genetics in the dairy cattle production industry. It is accepted as a standard breeding tool due to the reduction of costs associated with bull maintenance, the avoidance of infectious diseases, and the increase of superior genetic material. At present, although cryopreserved semen is more frequently used than fresh semen, frozen-thawed semen shows a sperm recovery rate of about 50% of that of fresh semen (Muino et al. 2007; Ugur et al. 2019). Semen freezing and thawing result in the sperm plasma membrane damage, reducing the viability of cryopreserved sperm after thawing.

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The plasma membranes are enriched with polyunsaturated fatty acids (PUFA) which are known to contribute to membrane fluidity. The susceptibility of sperm to cold shock during semen freezing has been associated with high PUFA which differ among species (Blesbois et al. 2005; Chanapiwat et al. 2009; Fair et al. 2014; Ugur et al. 2019). The higher PUFA resulted in a better response of sperm to cryopreservation (Mandal et al. 2014). However it is also elucidated that abundant PUFA could offer a highly susceptible response to lipid peroxidation in the presence of reactive oxygen species (ROS) (Cerolini et al. 2006). Excess of ROS can impair the sperm function and leads to infertility of various types of semen, including that of humans (Agarwal et al. 2014), pigs (Awda et al. 2009), rams (Budai et al. 2014), birds (Khan 2011), and bulls (Ugur et al. 2019). An acceptable attempt to decrease the effect of ROS is therefore adding of freezing extender with a variety of antioxidants such as vitamin C, vitamin E, glutathione peroxidase, and cysteine for increasing the sperm quality in a variety of animal species (Yeste 2016; Ugur et al. 2019).

Sericin is a protein derived from the silkworm *Bombyx mori* and a natural macromolecular and water-soluble globular protein. It consists of 18 different amino acids with strong polar side groups such as hydroxyl, carboxyl, and amino groups (Tao et al. 2005). Most amino acids consist of serine (31%), glycine (19.1%), glutamic acid (4.4%), aspartic acid (17.8%), threonine (8%), and tyrosine (3.3%). Studies have shown that sericin can scavenge hydroxyl radicals, superoxide radicals, and 1,1-diphenyl-2-picarylhydrazyl radicals and is involved in lipid peroxidation (Kato et al. 1998).

The previous study observed that sericin supplementation to *in vitro* culture medium could enhance preimplantation development and bovine embryo quality by preventing oxidative stress (Isobe et al. 2012). It also improved nucleus maturation and embryo quality in a porcine model (Do et al. 2014). Apart from its antioxidative activity, cryoprotective properties have been reported. For example, it was reported that sericin supplementation to a freezing medium for cryopreservation of bovine embryos decreased biological contamination from serum or bovine serum albumin (Isobe et al. 2013). This study was conducted to examine the effects of sericin supplementation for serum-free freezing medium on the survival and development of bovine embryos after freezing-thawing and direct transfer to recipients. When *in vitro*-produced bovine embryos were frozen conventionally in the freezing medium supplemented with various concentrations (0.1%, 0.5%, and 1.0%). In sperm cells, sericin supplementation to an extender improved the quality of human, buffalo bull, goat, and boar frozen-thawed semen by protecting sperm from oxidative stress (Kumar et al. 2015; Aghaz et al. 2018; Reddy et al. 2018; Ratchamak et al. 2020). However, information about the use of sericin in the cryopreservation of dairy bull semen has never been reported. In this context, we investigated the effects of addition of different concentrations of sericin in a semen freezing extender on sperm quality and malondialdehyde (MDA) levels of frozen-thawed semen in Holstein-Friesian crossbred bulls.

MATERIAL AND METHODS

Chemicals

All reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated.

Experimental animals

Five healthy dairy bulls (98% Holstein-Friesian crossbred) were used in the study. The dairy bulls, aged between three and six years, received a total mixed ration of 15.10 kg/day of concentrate with 14% protein; water was provided *ad libitum*.

Semen collection

Twenty-five ejaculates from five dairy bulls were assessed (five trials per animal). Semen was collected once a week from each bull for five consecutive weeks, using an electroejaculator (ElectroJac IV; Neogen, Lexington, KY, USA). Within 15 min after collection, the progressive motility was evaluated using a phase-contrast microscope at 400×. Sperm viability was evaluated by staining the sperm with eosin-nigrosin. Sperm concentrations were measured using a haemocytometer after the semen had dissolved and was immobilised with 4% sodium chloride. Only ejaculates with sperm motility and

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viability above 70% and concentration of more than 800×10^6 spermatozoa (spz)/ml were used for cryopreservation.

Semen cryopreservation

Semen samples were split into 15-ml centrifuge tubes and kept at 35 °C prior to dilution. Tris-egg yolk extender was used consisting of 1.675 g citric acid monohydrate, 1.25 g fructose, 3.028 g tris, and 0.1 g penomycin diluted with 100 ml ultrapure water and supplemented with 20% (vol/vol) egg yolk. The extender was supplemented with different concentrations of sericin (0, 0.25, 0.5, and 1.0% wt/vol). Each tube with extender was divided into two parts: one contained 14% glycerol and the other was without glycerol. Semen samples were diluted with the former extender at 35 °C, and the sperm concentration was adjusted to 120×10^6 spz/ml. Diluted semen was then cooled to 5 °C for about 2 h and then mixed with an equal volume of the latter extender, followed by equilibration at 5 °C for three hours. Subsequently, each diluted semen sample was loaded into a 0.5-ml plastic straw (IMV Technologies, L'Aigle, France) and sealed with polyvinyl powder; the final sperm concentration was 30×10^6 spz/straw. The straws were placed on a rack at 4 cm above the surface of liquid nitrogen in a Styrofoam box (25 × 35 × 30 cm) for 15 min and then plunged directly into liquid nitrogen. Semen was thawed in a water bath at 37 °C for 30 seconds.

Assessment of post-thaw sperm motility

The post-thaw motility of sperm was evaluated using Computer Assisted Sperm Analysis, CASA with IVOS v12.3 (Hamilton-Thorne, Beverly, MA, USA). An aliquot of 5 µl of frozen-thawed semen sample was placed in a 2X-CEL chamber slide (Hamilton-Thorne, Beverly, MA, USA) and prewarmed (37 °C) counting chamber and at least five fields were analysed. At least 300 spz/sample were analysed. The parameters of sperm motility consisted of sperm motility (MOT, %), progressive motility (PMOT, %), average pathway velocity (VAP, µm/s), straight line velocity (VSL, µm/s), curvilinear velocity (VCL, µm/s), amplitude of the lateral head displacement (ALH, µm), beat cross frequency (BCF, Hz), straightness (VSL/VAP ratio; STR, %), and linearity (VCL/VAP ratio; LIN, %).

Assessment of post-thaw sperm viability, acrosome integrity, and mitochondrial function

Post-thaw semen samples were evaluated for sperm viability, acrosome integrity and mitochondrial function by triple staining with propidium iodide (PI; Live/Dead® Sperm Viability Kit L7011; Invitrogen, Eugene, OR, USA), fluorescein isothiocyanate conjugated agglutinin from peanut (FITC-PNA) and 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazolyl-carbocyanine iodide (JC-1 Molecular Probes DMSO; Thermo Fisher Scientific, Waltham, MA, USA), respectively, as modified from Ratchamak et al. (2019). Briefly, 300 µl of post-thaw semen samples were diluted with 300 µl tris extender (without egg yolk and glycerol) at 37 °C. Diluted semen was mixed with 2 µl of PI, 2 µl of FITC-PNA (100 µg/ml, in PBS) and 5 µl of JC-1 (500 mM) and subsequently kept in the dark and incubated at 37 °C for 10 minutes. A total of 300 spermatozoa were randomly counted using a phase contrast epifluorescence microscope (Micromanipulator Olympus IX7; Olympus, Tokyo, Japan) in a triple filter set: UV-2E/C (excitation 340–380 nm and emission 435–485 nm), B-2E/C (excitation 465–495 nm and emission 515–555 nm), and G-2E/C (excitation 525–540 nm and emission 605–655 nm) at 400× magnification. With PI staining, the head of dead sperm appeared red and viable sperm remained colourless. Acrosome integrity was evaluated by FITC-PNA; yellow-green fluorescence indicated a damaged acrosome. In sperm with the high mitochondrial membrane potential, the midpiece region of sperm became fluorescent red-orange. The sperm was grouped in six categories as shown in Figure 1.

Determination of MDA concentration

The lipid peroxidation level was examined by measuring the production of MDA in semen. The MDA concentration was measured using thiobarbituric acid reactive substances (TBARS) as described by Ratchamak et al. (2019).

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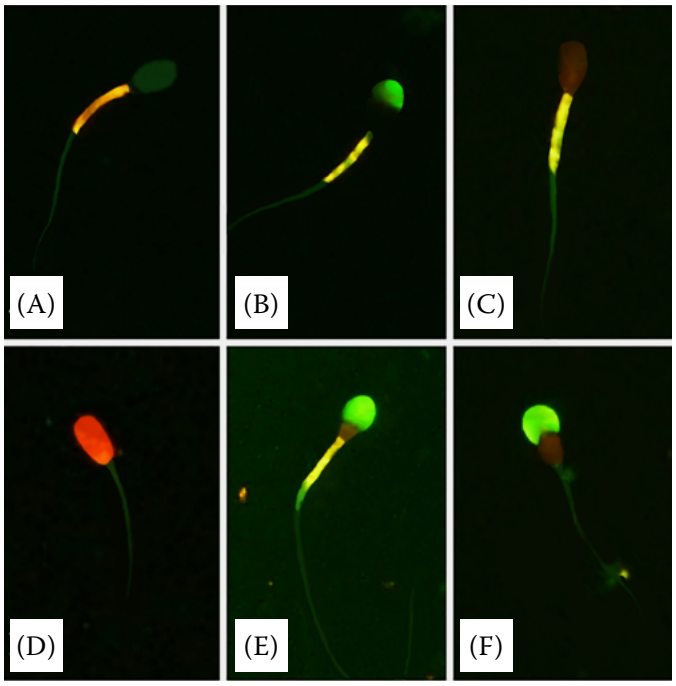

Figure 1. Fluorescence photomicrography of dairy bull spermatozoa stained with propidium iodide, fluorescein isothiocyanate conjugated agglutinin from peanut, and 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazolyl-carbocyanine iodide
Intact plasma and acrosomal membranes with high mitochondrial membrane potential (A); intact plasma membrane, damaged acrosome with high mitochondrial membrane potential (B); damaged plasma membrane, intact acrosome with high mitochondrial membrane potential (C); damaged plasma membrane, intact acrosome with low mitochondrial membrane potential (D); damaged plasma and acrosomal membranes with high mitochondrial membrane potential (E); damaged plasma and acrosomal membranes with low mitochondrial membrane potential (F)

Statistical analysis

A randomized complete block design was used in this study. Duncan's New Multiple Range Test was used to test differences in percentages of sperm quality and the MDA levels. Differences at $P < 0.05$ were considered statistically significant. The results were analysed using the statistical software program SAS/STAT® v9.0 software (SAS Institute Inc., Cary, NC, USA). The full statistical model was as follows:

$$y_{ij} = \mu + bull_i + ser_j + \varepsilon_{ij} \quad (1)$$

where:

y_{ij} – observation values of sperm motility, viability, acrosome integrity, mitochondrial function and MDA in treatment j (j = 1–4) at block i (i = 1–5);
μ – overall mean;
$bull_i$ – the effect of bull in block i;
ser_j – the effect of sericin concentrations in treatment j;
ε_{ij} – error term of the experiment.

Table 1. Effects of different sericin concentrations on sperm motility characteristics of frozen-thawed semen (mean ± SE)

Semen motion parameters	Sericin concentrations [% (wt/vol)]			
	0	0.25	0.5	1.0
Total motility (%)	35.62 ± 3.51^{b}	41.63 ± 3.56^{a}	40.66 ± 3.56^{a}	35.68 ± 3.31^{b}
Progressive motility (%)	20.43 ± 2.16	20.47 ± 1.90	20.78 ± 2.09	18.32 ± 2.09
VAP (μm/s)	75.09 ± 2.43	77.90 ± 2.30	78.07 ± 2.59	76.55 ± 2.59
VSL (μm/s)	59.27 ± 1.63	60.07 ± 1.77	60.16 ± 1.73	59.30 ± 1.74
VCL (μm/s)	132.61 ± 5.39^{b}	138.88 ± 4.66^{a}	138.54 ± 5.03^{a}	133.68 ± 4.87^{ab}
ALH (μm)	6.90 ± 0.14	6.88 ± 0.14	6.89 ± 0.15	6.83 ± 0.15
BCF (Hz)	24.38 ± 0.61	24.88 ± 0.67	24.89 ± 0.48	25.07 ± 0.59
STR (%)	76.17 ± 0.80	77.07 ± 0.83	77.62 ± 0.83	76.68 ± 0.48
LIN (%)	45.20 ± 0.94	45.59 ± 0.79	46.22 ± 0.81	46.23 ± 0.60

ALH = amplitude of lateral head displacement; BCF = beat cross frequency; LIN = linearity (ratio of VCL/VAP); STR = straightness (ratio of VSL/VAP); VAP = average pathway velocity; VCL = curvilinear velocity; VSL = straight line velocity
^{a,b}Different letters in the same row indicate statistical differences ($P < 0.05$)

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RESULTS

Fresh semen quality

The volume and concentration of fresh semen samples were 9.53 ± 0.77 ml and 1.05 ± 0.10 × 10^9 spz/ml, respectively. The mean percentages of sperm motility and viability were 83.67 ± 6.81% and 87.22 ± 5.53%, respectively.

Effect of sericin concentration on post-thaw semen quality

Results in Table 1 reveal that sericin supplemented in freezing extender at 0.25% and 0.5% enhanced the percentage of total sperm motility and curvilinear velocity of dairy bull semen after thawing ($P < 0.05$). Sericin had no effects on progressive motility, average pathway velocity, straight line velocity, ALH, BCF, STR, and LIN. However, the increasing sericin concentration to 1.0% had a negative effect on total motility as compared to other concentrations.

Data in Table 2 show that sericin supplemented in freezing extender at 0.25% resulted in higher percentages of live sperm with intact acrosomes and functioning mitochondrial post-thaw sperm compared to the control and 1.0% groups but did not differ from 0.5% groups ($P < 0.05$).

Effect of sericin concentration on lipid peroxidation

Results in Figure 2 document that sericin supplementation at 0.25% and 0.5% significantly decreased MDA concentrations compared with the control ($P < 0.05$), suggesting that an optimal level of sericin supplementation prevents oxidative damage to the frozen bull sperm.

DISCUSSION

Oxidative stress occurring during the freezing process causes excessive production of reactive oxygen species resulting in a pathological response and damage to sperm membranes, subsequently a decrease in sperm motility, sperm viability, membrane permeability and fertilization ability. The present study was therefore undertaken to establish a suitably modified semen diluent for improving the sperm quality of cryopreserved semen in dairy bulls. This is the first study to investigate the effect of addition of different concentrations of the antioxidant like sericin in semen freezing extender on sperm quality and MDA levels of frozen-thawed semen in Holstein-Friesian crossbred bulls.

Our findings show that the supplementation of freezing medium with 0.25% sericin was the most

Table 2. Effects of sericin concentrations on viability, acrosome integrity and mitochondrial function percentages of dairy bull sperm (mean ± SE)

Characteristics	Sericin concentrations [% (wt/vol)]			
	0	0.25	0.50	1.00
PI–/PNA–/JC1+	42.03 ± 2.17^{b}	48.29 ± 2.46^{a}	45.27 ± 2.50^{ab}	43.12 ± 2.57^{b}
PI–/PNA+/JC1+	3.80 ± 1.20	2.62 ± 0.86	3.82 ± 1.29	4.06 ± 1.13
PI+/PNA–/JC1+	21.71 ± 1.67	21.25 ± 1.77	22.12 ± 1.60	21.87 ± 1.42
PI+/PNA–/JC1–	3.53 ± 0.61^{a}	1.87 ± 0.33^{b}	2.56 ± 0.62^{ab}	1.72 ± 0.27^{b}
PI+/PNA+/JC1+	23.95 ± 2.01	22.43 ± 1.52	22.19 ± 1.89	25.22 ± 2.28
PI+/PNA+/JC1–	4.99 ± 1.11	3.53 ± 0.35	4.05 ± 0.66	4.02 ± 0.54

JC1 = 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazolyl-carbocyanine iodide; PI = propidium iodide; PNA = peanut agglutinin; PI–/PNA–/JC1+ = intact plasma and acrosomal membranes with high mitochondrial membrane potential; PI–/PNA+/JC1+ = intact plasma membrane, damaged acrosome with high mitochondrial membrane potential; PI+/PNA–/JC1+ = damaged plasma membrane, intact acrosome with high mitochondrial membrane potential; PI+/PNA–/JC1– = damaged plasma membrane, intact acrosome with low mitochondrial membrane potential; PI+/PNA+/JC1+ = damaged plasma and acrosomal membranes with high mitochondrial membrane potential; PI+/PNA+/JC1– = damaged plasma and acrosomal membranes with low mitochondrial membrane potential

[a,b]Different letters in the same row indicate statistical differences ($P < 0.05$)

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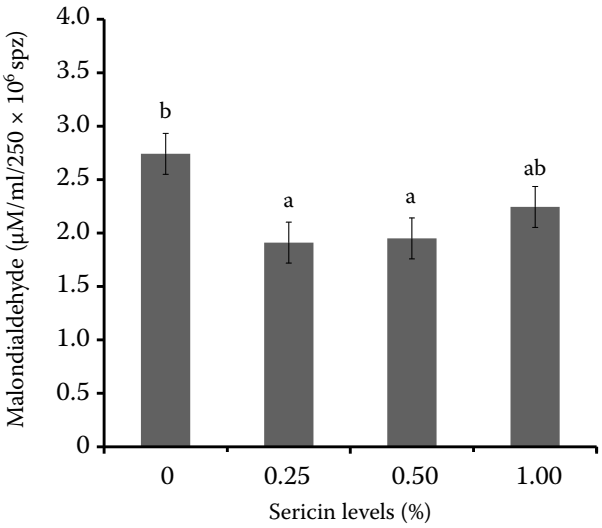

Figure 2. Effect of the addition of sericin to a freezing extender on malondialdehyde in semen cryopreservation from dairy bulls at different sericin levels (0%, 0.25%, 0.5%, and 1%), which are represented as bars (mean ± SE) Bars marked by different letters are significantly different ($P < 0.05$)

effective for improving the dairy bull semen quality in terms of sperm motility, viability, acrosome integrity and mitochondrial membrane potential while reducing the MDA concentration which is an indicator of lipid peroxidation. Results of another study indicated that 0.25–0.5% sericin supplementation improved the frozen-thawed buffalo bull semen quality by preventing oxidative stress (Kumar et al. 2015). Also, the addition of 2.5–5% sericin to a freezing and thawing medium successfully increased the total motility and viability in human sperm (Aghaz et al. 2018). This difference in concentration levels between studies might be due to the nature of extender, different species, and cryopreservation protocols.

The important factor regarding the damaged sperm plasma membrane after cryopreservation is an imbalance between antioxidants and free radicals occurring during the freezing procedure which can lead to oxidative stress and lipid peroxidation. Besides, the sperm plasma membrane is rich in polyunsaturated fatty acids, which are highly susceptible to the harmful effects of ROS, which decrease motility, morphology, plasma membrane integrity, and sperm fertility (Kefer et al. 2009). Normally the seminal plasma contains a variety of antioxidants for scavenging of ROS such as superoxide dismutase, catalase, and glutathione peroxidase. However, the antioxidants thoroughly decrease after the semen is collected as excessive ROS could be generated from dying ejaculated sperm as they are exposed to several potential sources of stress including dilution and cooling incubation during the freezing process (Ugur et al. 2019). For these reasons, the addition of a freezing extender containing an antioxidant becomes necessary. The results of the present study indicate that the sericin supplemented groups protected sperm against oxidative stress probably by reducing the amount of lipid peroxidation in 0.25–0.5% supplemented groups, subsequently providing a positive effect on post-thawed sperm quality. Those probably related to the structure of sericin contain high levels of hydroxyl amino acids such as serine and threonine (Yasmin et al. 2015). The hydrogen (H) atom effectively neutralizes hydroxyl radicals (•OH) in sperm cells, therefore the H atom markedly decreases oxidative stress. It has been reported that sericin addition can facilitate the cryopreservation of bovine embryos (Isobe et al. 2013), and it has been implied that sericin must exert a beneficial effect on any toxic occurring from cryopreservation resulting in the increased cell survival after freezing and thawing (Sasaki et al. 2005).

Sperm motility alone is an insufficient parameter for predicting fertility (Lange-Consiglio et al. 2013). The sperm motility is reliant on many considerations, for instance temperature, environment, or factors in laboratory procedures such as extender, types and sizes of slides and cover slips used (Quintero-Moreno et al. 2004), therefore a temporary motile sperm could occur after thawing. Other sperm parameters in terms of sperm viability, acrosome integrity and mitochondrial membrane potential represent the viable sperm which are important for fertilization. Thus those parameters were assessed at the same time using triple staining for predicting the cryopreserved sperm quality. The mitochondrial membrane potential is actually responsive to the energetic state of the sperm cell (Ly et al. 2003) and could be used as a potent indicator of sperm motility as it analysed mitochondrial integrity independently of a temporary motile sperm in spite of their transient immotility (Bussalleu et al. 2005). The results in this study show that the percentages of plasma membrane integrity, acrosome integrity and high mitochondrial potential were higher when compared with the percentages of sperm motility. Most of the mitochondrial sheaths were not damaged. Therefore

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the sperm with undamaged mitochondria could be definitely motile with any temporary immobility.

Interestingly, our study demonstrated that higher sericin supplementation at 1.0% negatively affected the sperm quality. This might be related to the higher lipid peroxidation in this group. Even though some studies showed that the scavenging activity of ROS was dose-dependent increasing with an increase in sericin concentration (Wu et al. 2007; Aghaz et al. 2018), however the best quality of post-thaw semen in our study was not observed at the highest sericin supplementation (1.0%). The higher concentration of antioxidants has been reported as toxic to sperm. For example, overdosing antioxidants led to a decrease in the functional integrity of the acrosome and plasma membrane (Atessahin et al. 2008). Higher DHA concentration promoted adverse effects on sperm motility (Losano et al. 2018). Lv et al. (2019) demonstrated that the higher amounts of resveratrol supplementation used did not inhibit the production of ROS and concurrently they did not diminish the cryodamage to goat sperm. Also, our finding was in accordance with Kumar et al. (2015), who reported that 1% to 2% sericin had harmful effects on the sperm cells. Although the addition of a freezing extender containing an antioxidant which acts as a peroxyl radical scavenger resulted in a balance between ROS production and neutralization (Bilodeau et al. 2000), the excessiveness of antioxidants may disturb the balance between free radicals and antioxidants in mammalian cells (Rahal et al. 2014). The adequate sericin doses in semen extender are therefore highly important.

CONCLUSION

In conclusion, a level of 0.25% (wt/vol) of sericin in a semen freezing extender seems to be optimal for enhancing the sperm quality of Holstein-Friesian crossbred bulls after thawing.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Agarwal A, Virk G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. World J Mens Health. 2014 Apr;32(1):1-17.

Aghaz F, Khazaei M, Vaisi-Raygani A, Bakhtiyari M. Cryoprotective effect of sericin supplementation in freezing and thawing media on the outcome of cryopreservation in human sperm. Aging Male. 2018 Nov 19: [8 p.].

Atessahin A, Bucak MN, Tuncer PB, Kizil M. Effects of antioxidant additives on microscopic and oxidative parameters of Angora goat semen following the freeze-thawing process. Small Rumin Res. 2008 Jun;77(1):38-44.

Awda BJ, Mackenzie-Bell M, Buhr MM. Reactive oxygen species and boar sperm function. Biol Reprod. 2009 Sep;81(3):553-61.

Bilodeau JF, Chatterjee S, Sirard MA, Gagnon C. Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. Mol Reprod Dev. 2000 Mar;55(3):282-8.

Blesbois E, Grasseau I, Seigneurin F. Membrane fluidity and the ability of domestic bird spermatozoa to survive cryopreservation. Reproduction. 2005 Mar;129(3):371-8.

Budai C, Egerszegi I, Olah J, Javor A, Kovacs A. The protective effect of antioxidants on liquid and frozen stored ram semen. Sci Pap Anim Sci Biotechnol. 2014 May 31; 47:46-52.

Bussalleu E, Pinart E, Yeste M, Briz M, Sancho S, Garcia-Gil N, Badia E, Bassols J, Pruneda A, Casas I, Bonet S. Development of a protocol for multiple staining with fluorochromes to assess the functional status of boar spermatozoa. Microsc Res Tech. 2005 Dec 1;68(5):277-83.

Cerolini S, Zaniboni L, Maldjian A, Gliozzi T. Effect of docosahexaenoic acid and α-tocopherol enrichment in chicken sperm on semen quality, sperm lipid composition and susceptibility to peroxidation. Theriogenology. 2006 Sep 1;66(4):877-86.

Chanapiwat P, Kaeoket K, Tummaruk P. Effects of DHA-enriched hen egg yolk and L-cysteine supplementation on quality of cryopreserved boar semen. Asian J Androl. 2009 Sep;11(5):600-8.

Do L, Namula Z, Luu V, Sato Y, Taniguchi M, Isobe T, Kikuchi K, Otoi T. Effect of sericin supplementation during in vitro maturation on the maturation, fertilization and development of porcine oocytes. Reprod Domest Anim. 2014 Apr;48(2):17-20.

https://doi.org/10.17221/104/2020-CJAS

Fair S, Doyle DN, Diskin MG, Hennessy AA, Kenny DA. The effect of dietary n-3 polyunsaturated fatty acids supplementation of rams on semen quality and subsequent quality of liquid stored semen. Theriogenology. 2014 Jan 15;81(2):210-9.

Isobe T, Ikebata Y, Onitsuka T, Wittayarat M, Sato Y, Taniguchi M, Otoi T. Effect of sericin on preimplantation development of bovine embryos cultured individually. Theriogenology. 2012 Sep 1;78(4):747-52.

Isobe T, Ikebata Y, Onitsuka T, Do LTK, Sato Y, Taniguchi M, Otoi T. Cryopreservation for bovine embryos in serum-free freezing medium containing silk protein sericin. Cryobiology. 2013 Oct;67(2):184-7.

Kato N, Sato S, Yamanaka A, Yamada H, Fuwa N, Nomura M. Silk protein, sericin, inhibits lipid peroxidation and tyrosinase activity. Biosci Biotechnol Biochem. 1998 Jan; 62(1):145-7.

Kefer JC, Agarwal A, Sabanegh E. Role of antioxidants in the treatment of male infertility. Int J Urol. 2009;16(5):449-57.

Khan RU. Antioxidants and poultry semen quality. Worlds Poult Sci J. 2011 Jun;67(2):297-308.

Kumar P, Kumar D, Sikka P, Singh P. Sericin supplementation improves semen freezability of buffalo bulls by minimizing oxidative stress during cryopreservation. Anim Reprod Sci. 2015 Jan;152:26-31.

Lange-Consiglio A, Meucci A, Cremonesi F. Fluorescent multiple staining and CASA system to assess boar sperm viability and membranes integrity in short and long-term extenders. Open Vet J. 2013 Mar;3(1),21-35.

Losano JDA, Angrimani DSR, Rui BR, Bicudo LC, Dalmazzo A, Silva BCS, Viana CHC, Mendes CM, Assumpcao MEOA, Barnabee VH, Nichi M. The addition of docosahexaenoic acid (DHA) and antioxidants (glutathione peroxidase and superoxide dismutase) in extenders to epididymal sperm cryopreservation in bulls. Zygote. 2018 Jun;26(3):199-206.

Lv C, Larbi A, Wu G, Hong Q, Quan G. Improving the quality of cryopreserved goat semen with a commercial bull extender supplemented with resveratrol. Anim Reprod Sci. 2019 Sep;208:106-27.

Ly JD, Grubb DR, Lawen A. The mitochondrial membrane potential (Δψm) in apoptosis; An update. Apoptosis. 2003 Mar;8(2):115-28.

Mandal R, Badyakar D, Chakrabarty J. Role of membrane lipid fatty acids in sperm cryopreservation. Adv Androl. 2014;2014: [9 p.].

Muino R, Fernandez M, Pena AI. Post-thaw survival and longevity of bull spermatozoa frozen with an egg yolk-based or two egg yolk-free extenders after an equilibration period of 18 h. Reprod Domest Anim. 2007 Jun;42(3):305-11.

Quintero-Moreno A, Rigau T, Rodriguez-Gil JE. Regression analyses and motile sperm subpopulation structure study as improving tools in boar semen quality analysis. Theriogenology. 2004 Feb;61(4):673-90.

Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, Dhama K. Oxidative stress, prooxidants, and antioxidants: The interplay. Biomed Res Int. 2014 Jan;2014: [19 p.].

Ratchamak R, Vongpralub T, Boonkum W, Chankitisakul V. Cryopreservation and quality assessment of boar semen collected from bulk samples. Vet Med-Czech. 2019 May 28;64(5):209-16.

Ratchamak R, Ratsiri T, Kheawkanha T, Vongpralub T, Boonkum W, Chankitisakul V. Evaluation of cryopreserved boar semen after supplementation sericin form silkworm (Bombyx mori) in semen extender. Anim Sci J. 2020 Jan-Dec;91(1):e13428.

Reddy VS, Yadav B, Yadav CL, Anand M, Swain DK, Kumar D, Kritania D, Madan AK, Kumar J, Yadav S. Effect of sericin supplementation on heat shock protein 70 (HSP70) expression, redox status and post thaw semen quality in goat. Cryobiology. 2018 Oct;84:33-9.

Sasaki M, Kato Y, Yamada H, Terada S. Development of a novel serum-free freezing medium for mammalian cells using the silk protein sericin. Biotechnol Appl Biochem. 2005 Oct;42(2):183-8.

Tao W, Li M, Xie R. Preparation and structure of porous silk sericin materials. Macromol Mater Eng. 2005 Mar; 290(3):188-94.

Ugur MR, Saber Abdelrahman A, Evans HC, Gilmore AA, Hitit M, Arifiantini RI, Purwantara B, Kaya A, Memili E. Advances in cryopreservation of bull sperm. Front Vet Sci. 2019 Aug 27;6: [15 p.].

Yasmin C, Otoi T, Setiadi MA, Karja NWK. Maturation and fertilisation of sheep oocytes cultured in serum-free medium containing silk protein sericin. Acta Vet Hung. 2015 Mar 1;63(1):110-7.

Yeste M. Sperm cryopreservation update: Cryodamage, markers, and factors affecting the sperm freezability in pigs. Theriogenology. 2016 Jan 1;85(1):47-64.

Wu JH, Wang Z, Xu SY. Preparation and characterization of sericin powder extracted from silk industry wastewater. Food Chem. 2007 Oct; 103(4): 1255–62.

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