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L-cysteine prolonged fresh boar semen qualities, but not for docosahexaenoic acid

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Abstract: The present study aimed to develop a long-term fresh boar semen extender by adding L-cysteine and docosahexaenoic acid (DHA) into Beltsville thawing solution (BTS)-based extender. Semen samples were collected from six boars, diluted at a concentration of 3×10^9 spermatozoa/100 ml and allocated into 10 groups as follows: BTS, Merck III®, Androstar® Plus, Modena™, DHA 1.5 mg/ml, 3 mg/ml and 6.25 mg/ml, L-cysteine 2.5 mM, 5.0 mM and 10 mM. All extended semen samples were stored at 18 °C, and were evaluated for progressive motility, viability, acrosome integrity, pH and osmolality on days 0, 1, 3, 5 and 7 of storage. The results documented that on day 3, L-cysteine at 2.5 mM showed the significantly highest percentage (78.8%) of progressive motility, but not different from Modena™ (78.8%) and Androstar® Plus (71.3%) ($P < 0.05$). On day 5, L-cysteine at 2.5 mM maintained progressive motility and viability up to 73.8% and 77.9%, respectively, but not different from Modena™ (78.8% and 74.8%) and Androstar® Plus (70.0% and 76.8%) ($P < 0.05$). On day 7, superior progressive motility (71.3%, 77.5% and 72.5%) and viability (72.8%, 77.3% and 69.8%) were found for L-cysteine at 2.5 mM, Modena™ and Androstar® Plus groups, respectively, when compared to the other groups ($P < 0.01$). However, in the present study, DHA supplementation failed to maintain fresh boar semen qualities during storage. In conclusion, adding L-cysteine at a concentration of 2.5 mM in BTS-based extender is the optimal concentration for the preservation of fresh boar semen at 18 °C for seven days.

Keywords: antioxidant; artificial insemination; DHA; pig; semen extender; spermatozoa

Currently, artificial insemination (AI) is an essential practice in pig industry worldwide and over 95% of pig farms use artificial insemination instead of natural mating. Most of them use the fresh diluted semen with $3–5 \times 10^9$ spermatozoa per 80–100 ml, kept at 18 °C before artificial insemination (Weitze 1990; Althouse et al. 1998; Knox et al. 2008; Kaeoket et al. 2010a). A major advantage of using fresh or chilled semen is the stability of sperm fertility which is maintained even with low numbers of spermatozoa in the AI dose (De Ambrogi et al. 2006). However, if the planned storage time is shorter than 72 h, it would be preferable to use short-term extenders since this type of extender is less expensive and the reproductive outcomes within three days are similar to those of long-term extenders (Johnson et al. 2000; Estienne et al. 2007). However, for long distance transportation, a long-term semen extender is considered (Gadea 2003). Long-term extenders differ from short-term extenders in that long-term extenders comprise more buffering agents (such as HEPES, Tris and TES)

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and antioxidants (e.g. hypotaurine, cysteine, vitamin E, ascorbic acid and glutathione) (Gadea 2003; De Ambrogi et al. 2006) in which they are able to maintain sperm quality for 7–14 days under cold storage (Funahashi and Sano 2005).

The boar sperm plasma membrane contains high levels of polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) (Johnson et al. 1969; Parks and Lynch 1992; Maldjian et al. 2005). These PUFAs decrease dramatically when the spermatozoa are attacked by reactive oxygen species (ROS) due to lipid peroxidation (White 1993; Sikka et al. 1995; Vazquez and Roldan 1997). It is well documented that antioxidants have beneficial effects on sperm quality during cold storage since they minimize detrimental effects of ROS on the sperm plasma membrane and enhance sperm survival rate and fertility after storage (Jang et al. 2006; Kumaresan et al. 2009; Kaeoket et al. 2010b; Bansal and Bilaspuri 2011). There are many studies that attempted to develop a boar semen extender by supplementation of antioxidants including adenosine, lycopene, taurine, vitamin E, ascorbic acid, superoxide dismutase and glutathione (Alvarez and Storey 1995; Szczesniak-Fabianczyk et al. 2003; Uysal and Bucak 2007). Funahashi and Sano (2005) reported that l-cysteine is an effective antioxidant for preventing oxidative stress in fresh boar semen during cold storage at 10 °C. In addition, it has been reported that DHA from fish oil and its combination with l-cysteine improved frozen-thawed boar semen quality (Chanapiwat et al. 2009; Kaeoket et al. 2010a, c; Chanapiwat et al. 2012a). However, the effect of DHA and l-cysteine supplement in a short-term semen extender on sperm quality has not been studied. Therefore, the aim of the present study was to develop a long-term extender by modifying (adding DHA or l-cysteine) the composition of Beltsville thawing solution (BTS) and investigate the boar sperm quality in terms of maintaining the sperm progressive motility, viability and acrosome integrity during storage at 18 °C for seven days.

MATERIAL AND METHODS

This research project was approved by the Faculty of Veterinary Science-Animal Care and Use Committee (FVS-ACUC-Protocol No. MUVS-2015-09).

Animals

Six Duroc boars (n = 6) of different age (between nine months and 2.5 years) were used in this study. The boars were housed in a closed housing system with individual pens. Each boar was used for routine artificial insemination and fed a commercial diet twice daily along with *ad libitum* water via water nipples.

Semen collection

Semen was collected using the gloved hand method. After collection, semen volume, pH, sperm concentration and the percentage of progressive motility were determined. Semen concentration was measured using a spectrophotometer (SpermaCue™; Minitüb GmbH, Tiefenach, Germany). Only ejaculates with a volume of > 100 ml, sperm concentration of > 150×10^6 sperm/ml and sperm motility of > 70% were used in this experiment.

Semen extenders and semen preparation

The semen from each boar was diluted with different extenders to a concentration of 3×10^9 spermatozoa/100 ml as earlier described by Kaeoket et al. (2010a). Semen samples were divided into 10 groups as follows: the first four groups served as controls which were extended with commercial extenders consisting of BTS (short-term extender; Minitüb GmbH, Tiefenach, Germany), Merck III® (medium-term extender; Minitüb GmbH, Tiefenach, Germany), Androstar® Plus (long-term extender; Minitüb GmbH, Tiefenach, Germany) and Modena™ (long-term extender; Swine Genetics International Ltd., Iowa, USA). The other six groups served as treatment groups in which the first three groups were diluted with BTS-based extender and supplemented with three different levels of DHA (1.5 mg/ml, 3 mg/ml and 6.25 mg/ml) (PFF35 powder, containing DHA 120 mg/g of powder; Martek Biosciences Corporation, MD, USA) (Kaeoket et al. 2010c) and the remaining three groups were diluted with BTS supplemented with different concentrations of L-cysteine (2.5 mM, 5.0 mM and 10.0 mM). Finally, the fresh diluted boar semen samples were stored at 18 °C and transported by cell incubator (Micom

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control system 20Q; Continental Plastic Corp., Delavan, WI, USA) to semen laboratory, Faculty of Veterinary Science, Mahidol University.

Sperm evaluation

During the experiment, diluted semen samples were stored at 18 °C in a refrigerator for seven days and evaluated on days 0, 1, 3, 5 and 7. Sperm aliquots were taken from diluted semen samples and incubated at 37 °C for 20 min before sperm evaluation.

Sperm progressive motility

The subjective progressive motility was evaluated by dropping 7 µl of semen sample on a prewarmed microscopic slide (37 °C) and covered with 22 × 22 mm coverslip. After preparing, each semen sample was evaluated immediately under a phase contrast microscope at 100× and 400× magnification. The motility was determined in each of the five fields, starting at the edge of slides to the opposite edge and ending at its centre. The average of the motility was weighted according to the number of progressive motile spermatozoa in each field and expressed as percentage of sperm progressive motility (Kaeoket et al. 2008; 2012).

Osmolality and pH measurement

Osmolality was assessed using a single-sample osmometer (Fiske® Micro-Osmometer; Advanced Instruments Inc., Norwood, MA, USA). An acceptable range is 240–380 mOsm/kg (Weitze 1990). The pH value was evaluated using a pH meter (PH Metro ConsortC830; Consort bvba, Turnhout, Belgium) and a range should not be lower than 6.5 (Newth and Levis 1999; Gadea 2003).

Sperm viability (sperm plasma membrane integrity)

Sperm viability was assessed by SYBR-14/Ethidiumhomodimer-1 (EthD-1) (LIVE/DEAD® Sperm Viability Kit; Thermo Fisher Scientific, Waltham, MA, USA). Briefly, 10 µl of diluted semen were mixed in the Eppendorf tube with 2.7 µl of the working solution of SYBR-14 (10 µM in DMSO, final concentration of 0.54 µM) and 10 µl of 1.17 µM EthD-1. After incubation at 37 °C for 20 min, a total of 200 spermatozoa were assessed under fluorescent microscope at 400× magnification. Spermatozoa were classified into two types as live sperm with intact plasma membrane (stained green with SYBR-14) and dead sperm with damaged plasma membrane (stained red with EthD-1). The results were expressed as the percentage of live sperm with intact plasma membrane (Kaeoket et al. 2010c; Chanapiwat et al. 2012b).

Sperm acrosome integrity

Acrosome integrity was assessed using fluorescein isothiocyanate-labelled peanut (*Arachis hypogaea*) agglutinin (FITC-PNA) staining. Briefly, 10 µl of diluted semen was mixed with 10 µl of Ethidiumhomodimer-1 and incubated at 37 °C for 15 minutes. Five microlitres of the mixture was smeared on a glass slide and fixed with 95% ethanol for 30 seconds. Fifty microlitres of the FITC-PNA solution (100 µg/ml in phosphate buffer saline) was spread over each slide and incubated in a moist and dark chamber at 4 °C for 30 minutes. Lastly, the sample slide was rinsed with cold phosphate buffer saline and air-dried before assessment (Maxwell and Johnson 1997; Chanapiwat et al. 2009; 2012b). A total of 200 spermatozoa were assessed under fluorescent microscope at 1 000× magnification and classified as intact acrosome and non-intact acrosome. The results were scored as the percentage of live sperm with intact acrosome (Chanapiwat and Kaeoket 2015).

Statistical analyses

Data from the evaluation of sperm qualities were analysed using the general linear model (SPSS v20.0; SPSS Inc., Chicago, IL, USA) and expressed as mean ± SD. The treatment groups and the day of measurement were modelled according to the factorial experiments in complete randomized design. The effects of treatments on parameters were analysed using the analysis of variance (ANOVA) in GLM. When the GLM revealed a significant effect, mean values were compared by Duncan's multiple range test. Differences were considered significant with $P < 0.05$.

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RESULTS

On average, the volume, concentration and progressive motility of fresh boar semen were 202.8 ± 88.3 ml, 349.0 ± 114.4 × 10^6 sperm/ml and 73.8 ± 4.8%, respectively. The values of progressive motility, viability, acrosome integrity, osmolality and pH on days 0, 1, 3, 5 and 7 are presented in Tables 1–5, respectively. Most semen qualities on days 0 and 1 were in a normal range (Tables 1 and 2), but the percentage of progressive motility of DHA-treated groups was rapidly decreased and remained lower than for the other extenders throughout the experiment.

On day 3 (Table 3), comparing all treatment groups, the L-cysteine 2.5 mM treated group had the significantly highest percentage (78.8%) of progressive motility, but it did not differ from long-term extenders (Modena™ and Androstar® Plus) (P < 0.05).

On day 5 (Table 4), comparing all treatment groups, the L-cysteine 2.5 mM treated group had the significantly highest percentage of progressive motility (73.8%) and viability (77.9%) compared to the other treatment groups except the long-term extender groups (Modena™ and Androstar® Plus) (P < 0.05).

On day 7 (Table 5) there were only three extenders that showed a progressive motility of more than 70%, i.e. Modena™ (77.5%), Androstar® Plus (72.5%) and L-cysteine 2.5 mM treated group (71.3%) (P < 0.05). These three extenders also had a higher percentage of viability than the other groups (Modena™ = 77.3%, Androstar® Plus = 69.8%, and L-cysteine 2.5 mM treated group = 72.8%).

Table 1. The mean (± SD) percentages of progressive motility, viability, acrosome integrity, osmolality and pH in different extenders on day 0 (n = 6)

Parameters	BTS	Merck III®	Androstar® Plus	Modena™	DHA 1.5 mg/ml	DHA 3 mg/ml	DHA 6.25 mg/ml	L-cysteine 2.5 mM	L-cysteine 5 mM	L-cysteine 10 mM
Motility	62.5 ± 22.2^{b}	65.0 ± 11.0^{b}	75.0 ± 10.8^{a}	83.8 ± 2.5^{a}	61.3 ± 13.2^{b}	30.0 ± 21.2^{c}	20.0 ± 21.2^{c}	62.5 ± 16.1^{b}	63.8 ± 16.1^{b}	60.0 ± 20.0^{b}
Viability	84.0 ± 3.7^{ab}	76.1 ± 4.0^{bc}	86.6 ± 2.8^{ab}	85.8 ± 8.8^{ab}	87.9 ± 7.7^{a}	73.3 ± 11.8^{cd}	73.0 ± 3.9^{cd}	77.1 ± 10.9^{bc}	80.9 ± 5.3^{ab}	71.9 ± 4.0^{d}
Acrosome integrity	75.6 ± 17.7	69.5 ± 30.7	83.8 ± 12.0	66.9 ± 29.8	79.3 ± 8.3	70.4 ± 22.2	62.1 ± 21.7	80.8 ± 17.2	74.3 ± 24.2	78.1 ± 17.9
Osmolality	334.5 ± 19.5^{b}	368.0 ± 6.5^{c}	310.3 ± 7.5^{a}	306.3 ± 2.2^{a}	341.5 ± 8.9^{b}	340.8 ± 13.7^{b}	342.3 ± 8.5^{b}	341.0 ± 19.3^{b}	337.3 ± 9.6^{b}	335.3 ± 7.4^{b}
pH	8.3 ± 0.1	8.3 ± 0.1	8.3 ± 0.1	8.2 ± 0.1	8.1 ± 0.1	8.1 ± 0.1	8.2 ± 0.1	8.2 ± 0.1	8.2 ± 0.1	8.1 ± 0.1

BTS = Beltsville thawing solution; DHA = docosahexaenoic acid

$^{a-d}$Values with different superscript letters within the same row are significantly different (P < 0.05)

Table 2. The mean (± SD) percentages of progressive motility, viability, acrosome integrity, osmolality and pH in different extenders on day 1 (n = 6)

Parameters	BTS	Merck III®	Androstar® Plus	Modena™	DHA 1.5 mg/ml	DHA 3 mg/ml	DHA 6.25 mg/ml	L-cysteine 2.5 mM	L-cysteine 5 mM	L-cysteine 10 mM
Motility	60.0 ± 20.4^{bc}	58.8 ± 14.4^{bc}	65.0 ± 24.2^{b}	81.3 ± 4.8^{a}	41.3 ± 23.2^{cd}	30.0 ± 21.6^{d}	20.0 ± 15.8^{d}	62.5 ± 15.6^{b}	32.5 ± 17.1^{cd}	22.5 ± 16.6^{d}
Viability	75.6 ± 7.0	71.6 ± 2.2	78.1 ± 5.7	79.5 ± 8.6	79.3 ± 14.7	72.8 ± 8.5	69.4 ± 14.2	78.1 ± 5.8	69.3 ± 6.0	64.9 ± 7.7
Acrosome integrity	64.4 ± 17.3	67.4 ± 11.0	61.4 ± 8.9	75.5 ± 4.7	68.9 ± 17.9	62.6 ± 6.6	64.4 ± 10.6	84.3 ± 2.6	60.3 ± 9.5	76.0 ± 8.0
Osmolality	334.3 ± 17.3^{b}	363.8 ± 6.9^{c}	304.3 ± 2.2^{a}	312.0 ± 8.4^{a}	344.0 ± 8.5^{b}	340.3 ± 8.7^{b}	343.0 ± 9.1^{b}	334.5 ± 4.8^{b}	332.5 ± 10.4^{b}	342.5 ± 14.6^{b}
pH	8.3 ± 0.1^{a}	8.3 ± 0.1^{a}	8.3 ± 0.1^{a}	8.2 ± 0.1^{ab}	8.1 ± 0.1^{c}	8.1 ± 0.1^{bc}	8.1 ± 0.1^{bc}	8.2 ± 0.1^{abc}	8.1 ± 0.1^{bc}	8.1 ± 0.1^{bc}

BTS = Beltsville thawing solution; DHA = docosahexaenoic acid

$^{a-d}$Values with different superscript letters within the same row are significantly different (P < 0.05)

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Table 3. The mean (± SD) percentages of progressive motility, viability, acrosome integrity, osmolality and pH in different extenders on day 3 (n = 6)

Parameters	BTS	Merck III®	Androstar® Plus	Modena™	DHA 1.5 mg/ml	DHA 3 mg/ml	DHA 6.25 mg/ml	L-cysteine 2.5 mM	L-cysteine 5 mM	L-cysteine 10 mM
Motility	68.8 ± 9.5^{ab}	50.0 ± 26.8^{bc}	71.3 ± 7.5^{ab}	78.8 ± 2.5^{a}	27.5 ± 23.9^{cd}	17.5 ± 16.6^{d}	11.3 ± 19.3^{d}	78.8 ± 4.8^{a}	60.0 ± 12.3^{ab}	28.8 ± 16.5^{cd}
Viability	69.5 ± 10.5^{a}	53 ± 8.8^{b}	72.9 ± 8.3^{a}	79.6 ± 11.3^{a}	75.9 ± 2.6^{a}	72.75 ± 11.9^{a}	71.3 ± 8.2^{a}	76.0 ± 6.7^{a}	75.8 ± 15.1^{a}	70.0 ± 16.3^{a}
Acrosome integrity	78.5 ± 10.3	72.5 ± 5.1	74.0 ± 10.6	67.4 ± 19.6	74.0 ± 14.3	66.4 ± 11.3	63.9 ± 12.5	64.6 ± 13.4	62.1 ± 12.7	63.8 ± 11.8
Osmolality	333.0 ± 8.4^{b}	366.5 ± 11.6^{d}	301.8 ± 14.8^{a}	305.8 ± 10.6^{a}	337.8 ± 3.4^{bc}	340.5 ± 4.8^{bc}	348.8 ± 14.9^{c}	327.3 ± 10.6^{b}	329.5 ± 4.4^{b}	333.3 ± 5.4^{b}
pH	8.3 ± 0.1^{bc}	8.7 ± 0.2^{a}	8.4 ± 0.2^{b}	8.3 ± 0.1^{bc}	8.2 ± 0.1^{bc}	8.2 ± 0.1^{bc}	8.1 ± 0.1^{bc}	8.2 ± 0.1^{bc}	8.2 ± 0.1^{bc}	8.2 ± 0.2^{bc}

BTS = Beltsville thawing solution; DHA = docosahexaenoic acid

$^{a–d}$Values with different superscript letters within the same row are significantly different (P < 0.05)

Table 4. The mean (± SD) percentages of progressive motility, viability, acrosome integrity, osmolality and pH in different extenders on day 5 (n = 6)

Parameters	BTS	Merck III®	Androstar® Plus	Modena™	DHA 1.5 mg/ml	DHA 3 mg/ml	DHA 6.25 mg/ml	L-cysteine 2.5 mM	L-cysteine 5 mM	L-cysteine 10 mM
Motility	51.3 ± 4.4^{bc}	51.3 ± 15.5^{bc}	70.0 ± 7.1^{a}	78.8 ± 2.5^{a}	25.0 ± 24.5^{d}	10.0 ± 13.5^{d}	3.8 ± 2.5^{d}	73.8 ± 2.5^{a}	46.3 ± 14.9^{c}	45.0 ± 19.2^{c}
Viability	60.4 ± 3.1^{d}	61.5 ± 5.4^{cd}	76.8 ± 6.1^{ab}	74.8 ± 12.3^{abc}	69.8 ± 6.2^{abc}	59.3 ± 7.9^{d}	67.3 ± 5.3^{abc}	77.9 ± 7.1^{a}	68.0 ± 10.9^{abc}	63.6 ± 11.8^{bcd}
Acrosome integrity	54.1 ± 30.6	54.9 ± 32.2	69.4 ± 11.1	60.8 ± 19.4	43.4 ± 12.7	56.0 ± 19.3	57.9 ± 2.2	68.0 ± 17.9	55.4 ± 18.9	55.5 ± 19.2
Osmolality	337.5 ± 17.8^{b}	363.8 ± 9.8^{c}	307.8 ± 4.3^{a}	310.8 ± 6.7^{a}	342.0 ± 6.1^{b}	348.3 ± 18.5^{c}	342.8 ± 9.4^{b}	334.0 ± 3.8^{b}	335.5 ± 4.4^{b}	342.5 ± 15.8^{b}
pH	8.3 ± 0.2^{ab}	8.6 ± 0.1^{a}	8.3 ± 0.3^{ab}	8.1 ± 0.2^{b}	8.0 ± 0.3^{b}	8.2 ± 0.4^{b}	8.2 ± 0.3^{b}	8.2 ± 0.2^{b}	8.2 ± 0.3^{b}	8.1 ± 0.2^{b}

BTS = Beltsville thawing solution; DHA = docosahexaenoic acid

$^{a–d}$Values with different superscript letters within the same row are significantly different (P < 0.05)

Table 5. The mean (± SD) percentages of progressive motility, viability, acrosome integrity, osmolality and pH in different extenders on day 7 (n = 6)

Parameters	BTS	Merck III®	Androstar® Plus	Modena™	DHA 1.5 mg/ml	DHA 3 mg/ml	DHA 6.25 mg/ml	L-cysteine 2.5 mM	L-cysteine 5 mM	L-cysteine 10 mM
Motility	38.8 ± 8.5^{d}	51.3 ± 6.3^{cd}	72.5 ± 6.5^{ab}	77.5 ± 2.9^{a}	16.3 ± 16.5^{e}	7.5 ± 15.0^{e}	5.0 ± 10.0^{e}	71.3 ± 4.8^{ab}	57.5 ± 8.7^{bc}	46.3 ± 18.9^{cd}
Viability	63.5 ± 13.3^{abc}	63.4 ± 8.6^{abc}	69.8 ± 14.7^{ab}	77.3 ± 9.6^{a}	54.5 ± 13.7^{bcd}	40.4 ± 11.1^{d}	42.3 ± 17.9^{cd}	72.8 ± 12.8^{ab}	54.1 ± 14.1^{bcd}	66.0 ± 15.0^{ab}
Acrosome integrity	53.9 ± 15.2	49.4 ± 20.7	44.8 ± 25.8	60.8 ± 16.4	46.0 ± 10.2	45.5 ± 19.3	57.5 ± 7.9	48.0 ± 19.9	52.0 ± 17.7	45.3 ± 10.8
Osmolality	324.8 ± 9.6^{c}	381.0 ± 8.9^{d}	322.3 ± 20.4^{a}	344.8 ± 12.7^{ab}	357.0 ± 3.8^{c}	361.0 ± 10.2^{c}	356.0 ± 13.5^{c}	341.3 ± 13.6^{bc}	346.5 ± 9.9^{c}	357.0 ± 11.4^{c}
pH	7.9 ± 0.5	8.1 ± 0.7	7.9 ± 0.2	7.8 ± 0.3	7.9 ± 0.5	7.9 ± 0.6	7.8 ± 0.6	7.8 ± 0.3	7.9 ± 0.4	7.9 ± 0.4

BTS = Beltsville thawing solution; DHA = docosahexaenoic acid

$^{a–e}$Values with different superscript letters within the same row are significantly different (P < 0.05)

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For sperm acrosome integrity, there was no significant difference between the treatment groups for the entire storage period (Tables 1–5).

DISCUSSION

In this study, we compared ten extenders with regard to sperm motility, viability and acrosome integrity of boar sperm during storage at 18 °C for seven days. Our results clearly demonstrated that the supplement of L-cysteine at a concentration of 2.5 mM was able to maintain sperm motility and viability up to seven days, which is comparable with long-term extenders (Modena™ and Androstar® Plus). However, the addition of DHA at any concentration could not maintain the sperm motility and viability during the preservation period. Reasonably, BTS and Merck III® maintained the semen qualities up to three days as claimed by the manufacturer. However, no significant difference in acrosome integrity was found between the treatment groups.

The present results showed that BTS semen extender supplemented with 2.5 mM L-cysteine could enhance the sperm motility and viability similarly like long-term extenders. L-cysteine, an amino acid containing a sulphhydryl group, is a precursor of intracellular glutathione biosynthesis. L-cysteine has an intracellular antioxidant capacity to decrease the amount of ROS and inhibit lipid peroxidation during cool storage (Bansal and Bilaspuri 2011) while it also acts as a membrane stabilizer and capacitation inhibitor (Johnson et al. 2000). It has been demonstrated that the addition of L-cysteine also improved sperm viability and increased the sperm survival time of boar spermatozoa during liquid preservation (Szczesniak-Fabianczyk et al. 2003; Funahashi and Sano 2005). Nevertheless, it is worth noting that too a high concentration of L-cysteine (5 mM and 10 mM) may have a detrimental effect on chilled boar semen quality. Similarly, Gungor et al. (2017) also indicated that the addition of a higher concentration of cysteine (4 mM) could maintain the ram sperm quality during chilled storage for 48 h but the sperm viability was significantly decreased at 96 h of storage when compared to 2 mM cysteine. These findings might be due to the limitation of L-cysteine uptake by spermatozoa and the amount of ROS production during the liquid preservation period (Kaeoket et al. 2010b).

During storage at a low temperature, boar spermatozoa containing high levels of PUFAs are highly susceptible to lipid peroxidation, leading to the sperm plasma membrane damage and consequently reducing sperm motility and causing a loss of the sperm function (Kumaresan et al. 2009). In the present study, DHA failed to improve the boar semen quality, especially sperm motility, during preservation at 18 °C. In agreement with previous studies, the addition of n-3 PUFAs directly to semen extenders could not improve the motility and viability of bull sperm in both chilled and frozen-thawed semen (Abavisani et al. 2013; Kandelousi et al. 2013). On the contrary, there were reported protective effects of adding PUFAs to semen extenders on maintaining sperm physical properties and functional integrity in boars (Hossain et al. 2007), bulls (Kaka et al. 2015) and rams (Towhidi et al. 2013; Rateb 2018). In frozen-thawed boar semen, it was demonstrated that DHA improved motility, membrane integrity, and acrosome integrity in boar sperm when used alone and in combination with L-cysteine in a freezing extender (Chanapiwat et al. 2009; Kaeoket et al. 2010c). Recently, Jakop et al. (2019) found that the addition of unsaturated fatty acids (linolenic, linoleic, oleic, palmitoleic acid) improved the boar sperm viability of cold stored semen at 6 °C for seven days. The n-3 PUFAs including α-linoleic acid, docosahexaenoic acid and eicosapentaenoic acid act directly on the sperm membrane lipid composition to promote permeability, fluidity and fusogenicity characteristics which are related to the sperm plasma membrane integrity, sperm motility and sperm survival rate (Tran et al. 2017). Likewise, Maldjian et al. (2005) also demonstrated that boar sperm can take DHA from a freezing extender supplemented with DHA, which leads to an improvement in post-thaw sperm quality. In bull sperm, previous studies revealed that supplementing the semen extender with n-3 PUFAs improved fatty acids in the sperm cell membrane and increased the quality of chilled and frozen sperm (Towhidi and Parks 2012; Kaka et al. 2015). However, the higher level of PUFAs (100 ng/ml) or excess of PUFAs increases the production of ROS during cryopreservation, subsequent lipid peroxidation reaction, resulting in detrimental effects on sperm quality (Towhidi and Parks 2012). Therefore, the supplementation of PUFAs needs to be optimized for species, individual, concentration and preservation procedures

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(Tran et al. 2017; Jakop et al. 2019). In principle, using a combination of PUFAs and antioxidants with their optimal concentration could be beneficial for the improvement of the boar sperm quality during low-temperature preservation of sperm.

CONCLUSION

In conclusion, a supplement of L-cysteine at a concentration of 2.5 mM in BTS extender is able to maintain progressive motility and viability (70%) comparable to commercial long-term extenders (Modena™ and Androstar® Plus).

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Conflict of interest

The authors declare no conflict of interest.

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