Indian Journal of Animal Sciences **93** (9): 888–892, September 2023/Article
https://doi.org/10.56093/ijans.v93i9.116312

Comparative efficacy of certain extenders on preservation of liquid boar semen

J KAUR[1], A K SINGH[2]✉, S S DHINDSA[2], P SINGH[2] and S K DAS[2]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

Received: 1 October 2021; Accepted: 27 July 2023

ABSTRACT

This study was carried out to compare the efficacy of certain liquid semen extenders for boar semen preservation. Semen ejaculates (n=30) from three boars (one ejaculate/boar/week) stationed at University pig farm, Ludhiana were collected using dummy sow and extended (1:4) in lactose-sodium salt of EDTA-Egg yolk (LSEEY); glucose sodium salt of EDTA-potassium sodium tartrate-sodium citrate (GEPS); and Modena (MOD). The extended semen was kept at 17°C up to 120 h in a BOD incubator. The semen was assessed for various sperm characteristics and lipid peroxidation at 0, 24, 48, 72, 96 and 120 h of preservation. The results revealed significantly higher percentage of sperm motility, viability, plasma membrane integrity and acrosome integrity in semen extended with MOD than in GEPS and LSEEY at different preservation times. The mean percentage of *in vitro* capacitation/acrosome reacted spermatozoa was significantly higher in MOD as compared to GEPS and LSEEY at 96 h and 120 h of storage period. In all the extenders, the mean percentage of most sperm attributes decreased progressively as the storage period increased. Correspondingly, the MDA levels were lower in MOD than in their contemporary extenders at all hours of preservation. In conclusion, Modena exhibited improved sperm parameters and reduced oxidative stress for liquid preservation of boar semen.

Keywords: Boar semen, Extender, Liquid preservation, Sperm attributes

In swine, most inseminations (>99%) are performed with extended liquid semen (Bielas *et al*. 2017). Liquid preservation of boar semen stored at 16-20°C is preferred over frozen semen owing to improved reproductive efficiency and profitability (Waterhouse *et al.* 2004). However, in earlier studies (De Ambrogi *et al.* 2006, Frydrychová *et al.* 2010) using boar liquid semen following longer periods of storage led to compromised quality and subsequent reduced fertility *vis-à-vis* type of diluent used. The quality of extended semen decreases as the storage time increases (Waberski *et al.* 2011). Different extenders tried for extension and preservation of boar semen exhibited variable results in relation to the fertilizing ability of spermatozoa in different breeds (Mapeka *et al.* 2012). During liquid preservation, boar spermatozoa experience several alterations like reduced motility alongwith modifications in plasma membrane permeability, thereby decreasing fertility with increase in storage time. High polyunsaturated fatty acid content in the phospholipid bilayer of plasma membrane and low antioxidant capacity of seminal plasma are the key factors to explain high sensitivity of boar sperm toward oxidative impairment (Cerolini *et al.* 2000). The excessive generation of reactive oxygen species (ROS) by spermatozoa has been linked to lipid peroxidation (LPO) whose outcome includes leakage of intracellular enzymes, inhibition of respiration and loss of membrane architecture, causing impaired fertility and decreased function of preserved spermatozoa (Kumaresan *et al.* 2009). In developing nations like India, studies using extenders for swine semen storage and their effect on sperm assays and peroxidative changes are scarce and limited. Hence, the current study was undertaken to investigate the effectiveness of certain extenders on sperm attributes and oxidative stress during liquid preservation of boar semen.

Present address: [1]Civil Veterinary Hospital (CVH), Cheema, Barnala. [2]Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. ✉Corresponding author email: assengar2001@yahoo.co.in

MATERIALS AND METHODS

Animals and semen collection: The Institutional Animal Ethics Committee (IAEC) of Guru Angad Dev Veterinary and Animal Sciences University (GADVASU/2020/IAEC/54/18) approved the present study. The animals used in the present study were treated humanely and all the experimental procedures were consistent with the guidelines of IAEC. Semen ejaculates (n=30) were collected from three trained, apparently healthy adult Large White Yorkshire breeding boars of 2-3 years age stationed at pig farm, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The breeding boars were maintained under uniform management and feeding system and housed in individual natural-ventilated pens. Using gloved hand technique; ejaculates of semen were

collected from three boars (one ejaculate/boar/week) with the help of dummy sow in a sterilized pre-warmed (37ºC) thermos flask in the morning hours. Semen samples (neat semen) which had initial progressive motility ≥70% were selected for extension and preservation. The study was conducted from February through May. During the study period, the mean daily temperature and relative humidity were 23.65±2.7°C and 56.0±6.5%, respectively inside the shed.

Extension, evaluation, processing and preservation of semen: Each filtered ejaculate was randomly aliquoted into three sterile beakers and extended (1:4) with lactose-sodium salt of EDTA-Egg yolk (LSEEY; Chutia *et al.* 2014), glucose sodium salt of EDTA-potassium sodium tartrate-sodium citrate (GEPS; Chutia *et al.* 2014), Modena (MOD; Saikia *et al.* 2016) extenders at 37°C. Thereafter, the extended semen placed in three glass beakers was preserved at 17°C for 120 h in a BOD incubator. All extenders were freshly prepared and stored at 5°C in a refrigerator. On the day of use, 20% egg yolk was added in LSEEY extender. Just prior to use, all extenders were placed in water bath at 37°C for further extension, evaluation and preservation of semen.

Analysis of sperm parameters: In the extended semen at 0 h (immediately after extension), 24, 48, 72, 96 and 120 h of preservation, the functional sperm parameters.

Individual progressive motility: The sperm motility was assessed by placing 10 µl extended semen on a pre-warmed glass slide and assessed subjectively using a light microscope in three different fields. Motility was expressed as the percentage of progressively motile spermatozoa.

Sperm viability: Eosin-Nigrosin staining technique was used to determine the live sperm count. Exactly, 10 µl extended semen was mixed with 10 µl stain. A thin smear was prepared from the semen-stain mixture on a clean glass slide and examined at 100× using light microscopy. The sperm which did not stain were considered live whereas partially stained and/or completely stained sperm were considered dead. About 200 spermatozoa in different fields were counted and sperm viability was estimated in percentage.

Plasma membrane integrity: To determine the functional integrity of plasma membrane, 1.0 ml hypo-osmotic solution (100 mosm/L) and 100 µl extended semen sample was mixed and incubated at 37°C for one hour. After incubation, 10 µl semen was kept on a glass slide and examined under the high power magnification (400×) using bright-field microscopy. A total of 200 spermatozoa with curled and non-curled tails were counted and the plasma membrane integrity was expressed in percentage.

Acrosome integrity: Giemsa stain was used for assessment of acrosome integrity of spermatozoa. From the extended semen a smear was prepared and stained with solution containing 2 ml 0.1 M PBS, 3 ml Giemsa stain stock solution and 35 ml distilled water for 120 min. After staining, the slides were rinsed in distilled water, air dried and examined under oil immersion (100×). Around 200 sperms with intact acrosome and damaged acrosome (partially or totally) were counted in various fields. The acrosome integrity was calculated as:

$$\text{Acrosome integrity (\%)} = \frac{\text{No. of sperm with intact acrosome}}{\text{Total sperm}} \times 100$$

In vitro capacitation/acrosome reaction: Briefly, 250 µl extended semen was centrifuged at 1000 rpm for 5 min with 2 ml energy rich TALP media. Following centrifugation, the sperm suspension was again suspended in 0.5 ml energy rich TALP media and incubated at 37°C for 6 h. A thin smear of semen with working solution of Giemsa stain was prepared and examined for *in vitro* capacitation/acrosome reaction at 0 h and 6 h. A total of 200 spermatozoa were counted in different fields at 40× magnification using bright-field microscope. The acrosome displaying shedding, vesiculation and swelling were considered *in vitro* capacitated/acrosome-reacted.

Analysis of LPO: The estimation of LPO was done through analysis of malondialdehyde (MDA) in the preserved semen at 0, 24, 48, 72, 96 and 120 h. Briefly, extended semen (2 ml) was centrifuged at 3000 rpm for 2 min. The pellet was centrifuged twice at 3000 rpm for two minutes with PBS (*p*H 7.4). Following centrifugation, the pellet was re-suspended in 100 µl PBS to which 100 µl of 150 mM Tris HCL (*p*H 7.1) was added. The sperm suspension was incubated at 37°C for 20 min. After incubation, 0.5 ml of 10% trichloroacetic acid and 1.0 ml of 0.375% thiobarbituric acid were added to the sperm suspension and kept in boiling water bath at 100ºC for 20 min. After cooling, the mixture was centrifuged for 15 min at 5000 rpm. Finally, the absorbance in supernatant was read at 532 nm and MDA was determined as:

$$\text{MDA (µmole/}10^9\text{ sperm)} = \frac{\text{O.D.} \times \text{Volume of assay mixture}}{\text{Extinction coefficient} \times \text{Volume of sample}}$$

Statistical analysis: Data were analyzed using SPSS (Statistical Package for the Social Sciences, version 26) program. The data on motility, live sperm count, plasma membrane integrity, acrosome integrity and *in vitro* capacitation/acrosome reaction and LPO in different extenders at different hours of preservation were analyzed by one way analysis of variance (ANOVA). All the proportionality data were corrected using angular transformation. ANOVA between the extenders and comparison of means was carried out using Tukey test. The results were expressed in mean±SE. A confidence level of $P<0.05$ was considered significant in all analyses.

RESULTS AND DISCUSSION

In the extended semen, the results generated on percentage of different sperm attributes and LPO at all hours of storage are shown in Table 1.

Sperm motility in different extenders: The mean percentage of motile sperm were significantly higher

Table 1. Sperm characteristics (Mean±SE) in liquid semen extended in selected extenders

Extender	Preservation period (h)						Overall
	0	24	48	72	96	120	
Individual progressive motility (%)							
MOD	81.4±1.4$^{a!}$	69.7±0.9$^{a\#}$	60.4±0.7$^{a\wedge}$	52.5±1.4^{a*}	45.2±1.6$^{a@}$	19.7±0.5^{a+}	54.8±1.1^{a}
GEPS	71.2±1.9$^{b!}$	62.2±1.3$^{b\#}$	54.8±1.0$^{b\wedge}$	48.6±0.8^{b*}	39.7±1.0$^{b@}$	15.2±0.8^{b+}	48.6±1.1^{b}
LSEEY	53.5±1.1$^{c!}$	37.8±0.8$^{c\#}$	22.5±1.7$^{c\wedge}$	15.5±0.9^{c*}	10.1±0.7$^{c@}$	5.0±1.6^{c+}	24.1±1.1^{c}
Sperm viability (%)							
MOD	89.7±0.9$^{a!}$	84.8±0.4$^{a\#}$	76.6±0.5$^{a\wedge}$	67.3±0.3^{a*}	56.6±0.4$^{a@}$	51.3±1.2^{a+}	71.1±0.6^{a}
GEPS	84.4±0.7$^{b!}$	77.3±1.2$^{b\#}$	72.2±1.0$^{b\#}$	62.3±0.4$^{b\wedge}$	51.0 ±1.0^{b*}	45.2±1.6$^{b@}$	65.4±1.0^{b}
LSEEY	58.1±1.6$^{c!}$	52.1±0.5$^{c\#}$	51.8±0.6$^{c\#}$	41.2±0.8$^{c\wedge}$	34.8±0.6^{c*}	25.3±1.4$^{c@}$	43.9±0.9^{c}
Sperm plasma membrane integrity (%)							
MOD	77.4±1.0$^{a!}$	63.6±0.8$^{a\#}$	56.9±1.1$^{a\wedge}$	50.1±0.6^{a*}	45.8±1.3$^{a@}$	39.6±1.6^{a+}	55.6±1.1^{a}
GEPS	69.1±1.4$^{b!}$	58.8±1.6$^{b\#}$	52.2±1.7$^{b\wedge}$	45.2±0.9^{b*}	37.8±0.9$^{b@}$	30.8±0.8^{b+}	48.9±1.2^{b}
LSEEY	47.2±0.5$^{c!}$	36.6±0.7$^{c\#}$	31.1±1.9$^{c\wedge}$	21.6±0.8^{c*}	10.4±0.7$^{c@}$	6.7±1.4^{c+}	25.6±1.0^{c}
Sperm acrosome integrity (%)							
MOD	92.4±1.2$^{a!}$	84.6±1.3$^{a\#}$	81.1±0.8$^{a\wedge}$	77.2±0.6^{a*}	73.2±1.8$^{a@}$	67.0±0.9^{a+}	79.3±1.1^{a}
GEPS	85.7±0.9$^{b!}$	80.0±1.7$^{b\#}$	77.6±0.5$^{b\wedge}$	73.1±0.9^{b*}	67.0±1.4$^{b@}$	63.0±0.6^{b+}	74.4±1.0^{b}
LSEEY	77.2±0.8$^{c!}$	73.5±0.9$^{c\#}$	68.7±1.3$^{c\wedge}$	63.7±1.0^{c*}	59.8±1.6$^{c@}$	54.8±1.2^{c+}	66.3±1.1^{c}

Values having different superscripts between column (alphabets) and within row (symbols) differ significantly (P<0.05).

(P<0.05) in MOD as compared to GEPS and LSEEY throughout the storage period (Table 1). Likewise, the mean sperm motility was significantly higher (P<0.05) in GEPS than in LSEEY. In all extenders, the sperm motility decreased significantly (P<0.05) at 0, 24, 48, 72, 96 and 120 h of preservation. Previous studies (Chutia *et al.* 2014, Saikia *et al.* 2016) have also shown highest (P<0.05) sperm motility in MOD followed by GEPS and LSEEY. High sperm motility in MOD could be due to its specific composition. De Ambrogi *et al.* (2006) demonstrated that the presence of bovine serum albumin (BSA) in MOD strengthens the plasma membrane of spermatozoa and increases sperm motility. Further, presence of insoluble egg yolk granules, clumping and agglutination appeared to reduce the efficacy of LSEEY in preserving the sperm motility for a longer period of time (Chutia *et al.* 2014). Gradual loss in nutritional content seemed to be a probable factor which decreased sperm motility in all extenders with increased preservation time.

Sperm viability in different extenders: Amongst the three extenders, the sperm viability was significantly higher (P<0.05) in MOD than in GEPS and LSEEY during the storage period (Table 1). Similarly, the mean proportion of viable sperm were significantly (P<0.05) higher in semen extended with GEPS as compared to that with LSEEY during different times of preservation. At all storage times, the sperm viability decreased progressively during the study period in all the extenders. These observations are in accordance with the findings of Saikia *et al.* (2016) who noticed higher sperm viability in MOD than its contemporary extenders. MOD contains cysteine which owing to its antioxidant properties plays an important role in maintenance of sperm membrane through inhibition of LPO due to lesser generation of ROS (Kaeoket *et al.* 2010). LPO has been regarded as the major cause of cell membrane impairment leading to decreased viability of spermatozoa (Flores *et al*. 2008). Higher proportion of viable sperm in GEPS compared to LSEEY might be due to the presence of potassium sodium tartrate (Chutia *et al.* 2014).

Plasma membrane integrity in different extenders: Observations on 30 ejaculates revealed that MOD and GEPS maintained significantly (P<0.05) higher plasma membrane integrity as compared to LSEEY extender during the storage period. A similar trend (P<0.05) of higher percentage of sperm with intact membrane was seen in MOD than in GEPS. The sperm plasma membrane integrity decreased gradually from time of collection (0 h) until 120 h collection in all the extenders. Similar studies (Sangma *et al.* 2020) depicted higher proportion of intact plasma membrane in MOD than in GEPS and LSEEY throughout the study period (0-120 h). The decline in plasma membrane integrity could be due to decreased sperm biochemical activity in all extenders as the preservation time progressed. Kumaresan *et al.* (2009) demonstrated that abundance of unsaturated fatty acids and lack of antioxidant-rich cytoplasmic component contributes toward vulnerability of sperm membrane to LPO. As reported earlier, MOD contains BSA that plays a strategic role in decreasing LPO and subsequently reinforces the plasma membrane (De Ambrogi *et al.* 2006), thereby resulting in higher proportion of intact plasma membrane in the current study.

Acrosome integrity in different extenders: In the present study, the percentage of intact acrosome was significantly (P<0.05) higher in MOD as compared to that in GEPS and LSEEY at 0, 24, 48, 72, 96 and 120 h of preservation. Similarly, the acrosome integrity was significantly (P<0.05) higher in GEPS than in LSEEY throughout the storage period. Nevertheless, as observed in previous sperm attributes, the acrosome integrity decreased significantly

Table 2. Percentage of *in vitro* capacitation / acrosome reaction (Mean±SE) in liquid semen extended in different extenders

Extender	Acrosome reaction	Preservation period (h)						Overall
		0	24	48	72	96	120	
MOD	0 h	1.8±0.6*	1.9±0.8$^{\wedge *}$	2.4±0.7$^{\wedge *}$	3.5±0.9$^{!\#\wedge}$	4.1±0.8$^{!\#}$	5.4±0.7$^{!}$	3.2±0.7
	At 6 h	6.1±0.8*	6.9±0.9*	8.2±1.0$^{*\wedge}$	10.1±1.2$^{\wedge}$	19.2±1.2$^{a\#}$	24.8±1.2$^{a!}$	12.5±1.1^{a}
	Final acrosome reaction (%)	4.3±0.2*	5.0±0.1*	5.8±0.3$^{*\wedge}$	6.6±0.3$^{\wedge}$	15.1±0.4$^{a\#}$	19.4±0.5$^{a!}$	19.4±0.3^{a}
GEPS	0 h	2.3±0.8$^{\#}$	2.7±0.7$^{\#}$	3.0±0.9$^{!\#}$	3.7±0.7$^{!\#}$	4.4 ±1.1$^{!\#}$	4.9±0.8$^{!}$	3.5±0.8
	At 6 h	6.9±0.9*	7.4±1.2$^{*\wedge}$	8.4±1.1$^{*\wedge}$	9.3±1.2$^{\wedge}$	15.8±1.3$^{b\#}$	18.6±1.2$^{b!}$	11.1±1.2^{b}
	Final acrosome reaction (%)	4.6±0.1*	4.7±0.5$^{*\wedge}$	5.4±0.2$^{*\wedge}$	5.6±0.5$^{\wedge}$	11.4±0.2$^{b\#}$	13.7±0.4$^{b!}$	13.7±0.3^{b}
LSEEY	0 h	1.2±0.7$^{\wedge}$	2.8±0.8$^{\wedge\#}$	3.3±1.2$^{\wedge\#}$	4.2±0.8$^{\#}$	5.0±0.9$^{\#!}$	6.2±0.7$^{!}$	3.8±0.9
	At 6 h	7.2±1.1*	7.1±0.9*	9.1±1.4$^{\wedge *}$	9.9±1.3$^{\wedge}$	16.1±1.2$^{b\#}$	18.2±0.9$^{b!}$	11.3±1.1^{b}
	Final acrosome reaction (%)	6.0±0.4*	4.3±0.1*	5.8±0.2$^{\wedge *}$	5.7±0.5$^{\wedge}$	11.1±0.3$^{b\#}$	12.0±0.2$^{b!}$	12.0±0.3^{b}

Values having different superscripts between column (alphabets) and within row (symbols) differ significantly (P<0.05).

(P<0.05) in all the extenders with increased storage time. Similar studies (Kaeoket *et al.* 2010, Chutia *et al.* 2014) have also shown the superiority of MOD over GEPS and LSEEY. In the current study, the proportion of spermatozoa with intact acrosome decreased as the preservation time increased in all extenders. A similar decrease in the proportion of spermatozoa with normal ultrastructure of acrosome was noticed by Frydrychová *et al.* (2010) due to high levels of cholesterol and phospholipid concentrations that can affect sperm acrosome during storage.

In vitro capacitation/acrosome reaction in different extenders: At 0 h of observation, no significant (P>0.05) difference was seen among the three extenders in respect of *in vitro* capacitated/acrosome reacted spermatozoa during the respective the storage time (Table 2). After 6 h of incubation, the *in vitro* capacitation/acrosome reaction was similar (P>0.05) in semen extended in MOD, GEPS and LSEEY at 0-72 h of storage. At 96 h and 120 h of preservation, the acrosome reaction was higher (P<0.05) in MOD as compared to that in GEPS and LSEEY after 6 h of incubation. In all the extenders, the *in vitro* capacitation/acrosome reaction increased at all storage times both at 0 h of observation and following 6 h of incubation. Comparative studies showing efficacy of the extenders selected in the present study on *in vitro* capacitation/acrosome reaction during liquid preservation of boar semen are not available in literature. Nonetheless, Conejo-Nava *et al.* (2003) showed that presence of sodium bicarbonate in MOD increases calcium influx, induces rapid and reversible organizational modifications of lipid bilayer of sperm membrane and stimulates a capacitation signal transduction pathway thereby initiating the acrosome reaction during storage. The authors further reported that sodium bicarbonate decreases membrane fluidity especially at the head region of spermatozoa as compared to other regions, leading to capacitation signal transduction pathway. In the current study, presence of sodium bicarbonate in MOD, might contribute toward fundamental changes in structural membrane of spermatozoa leading to better capacitation status.

LPO in different extenders: The mean levels of LPO as manifested by malondialdhyde (MDA) produced are presented in Table 3. Although non-significant (P>0.05), the MDA profiles were lower in MOD than in its counterparts at 0 h of preservation. At 24 and 48 h of storage period, the levels of MDA were significantly (P<0.05) lower in the semen preserved in MOD as compared to that in GEPS and LSEEY. No difference (P>0.05) in MDA production was noticed between GEPS and LSEEY at 24 and 48 h of preservation. At 72-120 h of preservation, the MDA concentrations were significantly (P<0.05) reduced in MOD than in GEPS and LSEEY. Likewise, significantly (P<0.05) lower levels of MDA were recorded in semen extended in GEPS as compared to that in LSEEY at 72-120 h of storage period. However, the levels of MDA increased exponentially (P<0.05) in all the extenders at all hours of preservation. This is the first study of its kind to compare MDA profiles in semen extended with MOD, GEPS and LSEEY extenders. Limited comparative studies (Bucak *et al.* 2010) in other extenders (Beltsville thawing solution and Safe Cell) revealed that MDA concentrations increased considerably (P<0.05) as the duration of preservation increased (Karunakaran *et al.* 2017). The ROS-induced sperm destruction triggered the LPO cascade leading to loss of membrane integrity, decreased cell function and ultimately sperm death. In MOD, lower MDA levels could be due to the impact of cysteine and BSA on free radicals, thereby, preventing the plasma

Table 3. Lipid peroxidation (MDA, µmole/10^9 sperm) levels (Mean±SE) in liquid semen extended in selected extenders

Extender	Preservation period (h)						Overall
	0	24	48	72	96	120	
MOD	0.27±0.06$^{\wedge}$	0.57±0.04$^{b\#}$	0.58±0.05$^{b\#}$	0.60±0.06$^{c\#}$	0.78±0.07$^{c!}$	0.88±0.02$^{c!}$	0.61±0.05^{c}
GEPS	0.41±0.08$^{\wedge}$	0.74±0.03$^{a\#}$	0.78±0.09$^{a\#}$	0.79±0.05$^{b\#}$	1.01±0.09$^{b!}$	1.18±0.08$^{b!}$	0.82±0.07^{b}
LSEEY	0.33±0.05*	0.70±0.06$^{a\wedge}$	0.77±0.08$^{a\wedge}$	1.15±0.08$^{a\#}$	1.18±0.04$^{a!\#}$	1.41±0.04$^{a!}$	0.92±0.06^{a}

Values having different superscripts between column (alphabets) and within row (symbols) differ significantly (P<0.05).

membrane damage (De Ambrogi *et al.* 2006, Kaeoket *et al.* 2010). Eventually, in the present study, cysteine and BSA component of MOD might have lowered MDA production leading to reduced peroxidation effect to sperm cells.

Thus, it can be concluded that liquid preservation of boar semen in Modena extender provides better sperm attributes and protection to plasma membrane as well as reduced oxidative stress.

ACKNOWLEDGEMENTS

The authors are thankful to the Indian Council of Agricultural Research for providing required funding under the project entitled "All India Coordinated Research Project on Pig" to carry out the research work.

REFERENCES

Bielas W, Niżański W, Partyka A, Rząsa A and Mordak R. 2017. Effect of long-term storage in Safe Cell+ extender on boar sperm DNA integrity and other key sperm parameters. *Acta Veterinaria Scandinavica* **59**(1): 1–12.

Bucak M N, Sarıözkan S, Tuncer P B, Sakin F, Ateşşahin A, Kulaksız R and Çevik M. 2010. The effect of antioxidants on post-thawed Angora goat (*Capra hircus ancryrensis*) sperm parameters, lipid peroxidation and antioxidant activities. *Small Ruminant Research* **89**(1): 24–30.

Cerolini S, Maldjian A, Surai P and Noble R. 2000. Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage. *Animal Reproduction Science* **58**(1): 99–111.

Chutia T, Biswas R K, Tamuli M K, Sinha S, Goswami J, Deka B C, Banik S and Kayastha R B. 2014. Efficacy of different extenders in preservation of liquid hampshire boar semen at 15°C. *Indian Journal of Animal Research* **48**(5): 496–500.

Conejo-Nava J, Fierro R, Gutierrez C G and Betancourt M. 2003. Membrane status and *in vitro* capacitation of porcine sperm preserved in long-term extender at 16°C. *Archives of Andrology* **49**(4): 287–95.

De Ambrogi M, Ballester J, Saravia F, Caballero I, Johannisson A, Wallgren M, Anderson M and Rodriguez-Martinez H. 2006. Effect of storage in short-and long-term commercial semen extenders on the motility, plasma membrane and chromatin integrity of boar spermatozoa. *International Journal of Andrology* **29**(5): 543–52.

Flores E, Cifuentes D, Fernández-Novell J M, Medrano A, Bonet S, Briz M D and Rodríguez-Gil J E. 2008. Freeze-thawing induces alterations in the protamine-1/DNA overall structure in boar sperm. *Theriogenology* **69**(9): 1083–94.

Frydrychová S, Čeřovský J, Lustyková A and Rozkot M. 2010. Effects of long-term liquid commercial semen extender and storage time on the membrane quality of boar semen. *Czech Journal of Animal Science* **55**(5): 160–66.

Kaeoket K, Srisowanna T, Wichaidit U, Chanapiwat P and Manee S. 2010. Comparative study on six different long term commercial extenders for fresh boar semen. *The Thai Journal of Veterinary Medicine* **40**(3): 257–63.

Karunakaran M, Chakurkar E B, Ratnakaran U, Naik P K, Mondal M, Mondal A and Singh N P. 2017. Characteristics of boar semen preserved at liquid state. *Journal of Applied Animal Research* **45**(1): 217–20.

Kumaresan A, Kadirvel G, Bujarbaruah K M, Bardoloi R K, Das A, Kumar S and Naskar S. 2009. Preservation of boar semen at 18ºC induces lipid peroxidation and apoptosis like changes in spermatozoa. *Animal Reproduction Science* **110**(1): 162–71.

Mapeka M H, Lehloenya K C and Nedambale T I. 2012. Comparison of different extenders and storage temperature on the sperm motility characteristics of Kolbroek pig semen. *South African Journal of Animal Science* **42**(5): 530–34.

Saikia T, Ahmed K, Tamuli M K and Ahmed N. 2016. Effect of different extenders on the quality of boar semen during preservation at 15°C and 5°C. *Theriogenology Insight* **6**(1): 73–77.

Sangma T F M, Ahmed K, Choudhury M D, Zaman G U, Ahmed N and Das A. 2020. Comparative efficacy of three extenders on quality of boar semen during preservation at 15°C. *Indian Journal of Animal Sciences* **90**(3): 375–78.

Waberski D, Henning H and Petrunkina A M. 2011. Assessment of storage effects in liquid preserved boar semen. *Reproduction in Domestic Animals* **46**(2): 45–48.

Waterhouse K E, De Angelis P M, Haugan T, Paulenz H, Hofmo P and Farstad W. 2004. Effects of *in vitro* storage time and semen-extender on membrane quality of boar sperm assessed by flow cytometry. *Theriogenology* **62**(9): 1638–51.