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Efficacy of different extenders on sperm characteristics and lipid peroxidation during liquid storage of boar semen

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ABSTRACT

The present study was aimed to test and evaluate the efficacy of six different extenders, Modena, Beltsville thawing solution (BTS), glucose sodium salt of EDTA-potassium sodium tartrate-sodium citrate (GEPS), Safe Cell, Androstar Plus (ASP) and M III on boar sperm parameters and lipid peroxidation during preservation. Sixty ejaculates were collected from six breeding Large White Yorkshire boars and preserved at 17°C for 120 h. Each ejaculate was evaluated for motility, viability, hypo-osmotic swelling test (HOST), acrosome integrity, *in vitro* capacitation/acrosome reaction and lipid peroxidation based on malonaldehyde (MDA) levels at 0, 24, 48, 72, 96 and 120 h of storage. The percentage of motile and viable spermatozoa, HOS-positive sperm population, intact acrosomes and *in vitro* capacitated/acrosome-reacted spermatozoa declined significantly from 0-120 h of preservation in all extenders. BTS and Modena extenders preserved higher sperm motility and viability from 24-120 h and acrosome integrity from 48-120 h of storage in comparison to remaining extenders. BTS maintained highest proportion of HOS-positive sperm at 96 h and 120 h and *in vitro* acrosome reaction percentage at 120 h of preservation among all extenders. From 24-120 h of storage, the MDA concentrations were lower in semen preserved in BTS and Modena than the other extenders. Thus, it can be concluded that both BTS and Modena were more effective among the tested extenders for preservation of boar semen at 17°C.

Keywords: Boar semen, Extenders, Liquid preservation, Semen analysis

In swine industry, most inseminations (>99%) are performed with extended liquid semen (Johnson *et al.* 2000). Liquid preservation of boar semen at 16-20°C is preferred over frozen semen whether used on the same day and/or within 24 h post-collection (Karunakaran *et al.* 2017). However, the main barrier to AI development using semen doses 24 h following extension is compromised fertility, especially in terms of litter size (Christensen *et al.* 2004). While few studies (De Ambrogi *et al.* 2006, Frydrychová *et al.* 2010, Roca *et al.* 2011) have shown poor semen quality following long storage periods (after 72-96 h) irrespective of the type of diluent used, others (Boe-Hansen *et al.* 2008, Bielas *et al.* 2017) reported no effect on sperm characteristics. These inconsistencies between reported scientific literature along with growing interests to determine the optimal storage conditions for diluted boar spermatozoa demand a call for further research in this area.

Most extenders are prepared with the aim to increase their storage competency by providing nutrients for sperm metabolism, protection against cold shock, maintenance of osmotic balance, neutralize metabolic residue during storage, prevent *p*H alterations and inhibit bacterial growth (Mapeka *et al.* 2012, Henning *et al.* 2022). However, many weaknesses still persist in liquid preservation of boar semen which need to be improved prior to its routine use in commercial pig farms on a large scale. In developing nations like India, studies using extenders for storage of boar semen and their effect on sperm assays are scarce and limited. Hence, the present study was undertaken with the objective to evaluate the effectiveness of six different semen extenders on boar sperm attributes and lipid peroxidation during storage.

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MATERIALS AND METHODS

Semen collection, evaluation, processing, extension and storage: The present study was approved by the Institutional Animal Ethics Committee (IAEC) of the University (GADVASU/2020/IAEC/54/18-19) and all the experimental procedures were consistent with the guidelines of IAEC. Sixty ejaculates were collected from six Large White Yorkshire breeding boars (10 ejaculates per boar) maintained at pig farm, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana and at organized private pig farms located in the peri-urban areas (20 km drive from the university) of Ludhiana. All boars at both farms were maintained under uniform

management and feeding system and housed in individual natural-ventilated pens. The study was conducted during the months of February through April, 2022. During the study period, the mean daily temperature and relative humidity were 23.65±2.7°C and 56.0±6.5%, respectively inside both the sheds. Semen ejaculates from each boar were collected in a pre-warmed (37ºC) sterilized thermos flask using gloved hand technique in morning hours. Ejaculates (neat semen) having initial motility ≥70%, sperm concentration ≥250×10^6/mL, abnormal spermatozoa <20% and volume ≥200 mL were used in the current study. Each ejaculate was divided into six sterile beakers and extended (1:3 to 1:4) with six selected extenders including Modena (Khan *et al.* 2006), Beltsville thawing solution (BTS; Kumaresan *et al.* 2009), glucose sodium salt of EDTA-potassium sodium tartrate-sodium citrate (GEPS; Sangma *et al.* 2020), Safe Cell (Bielas *et al.* 2017), Androstar Plus (ASP; Frydrychová *et al.* 2010) and M III (Frydrychová *et al.* 2010). Following extension, the semen was packed in a plastic bottle (80 ml) using tube sealer machine (Minitüb Technologies, Germany) and stored at 17°C for 120 h in a BOD incubator.

The Safe Cell (IMV Technologies, L'Aigle, France), ASP and M III (Minitüb, Germany) were commercially available extenders and kept at 5°C. The Modena, BTS and GEPS extenders (*p*H 7.2) were prepared fresh 12 h prior to use and kept in a refrigerator at 5°C. On the day of use, egg yolk (20%) was added to GEPS extender. Immediately before use, all extenders were placed in water bath at 37°C for further extension and preservation of semen.

Evaluation of sperm characteristics

In the extended semen at 0 h (immediately after extension), 24, 48, 72, 96 and 120 h of preservation, the selected functional sperm attributes were estimated.

Sperm motility: The sperm motility was subjectively evaluated by placing 10 µL extended semen on a pre-warmed glass slide and assessed (40×) using a zoom stereo light microscope (Olympus Opto Systems, India) to the nearest 5% in three different fields and expressed in percentage.

Sperm viability: The live sperm count was determined using Eosin-Nigrosin staining technique (Bamba 1988). Exactly 10 µL semen was mixed with 10 µL stain. A thin smear was prepared immediately 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 calculated in percentage.

Hypo-osmotic swelling test (HOST): To determine the functional integrity of plasma membrane, 100 µL semen was mixed with 1.0 mL of hypo-osmotic solution (HOS; Pérez-Llano *et al.* 2003). Simultaneously, 1.0 mL of phosphate buffer saline (control) was added to 100 µL semen. The semen mixture in both HOS as well as control was incubated at 37°C for 1 h. After incubation, 10 µL semen mixed with HOS and control was placed on separate glass slides and observed using bright-field microscopy. About 200 spermatozoa with curled and non-curled tails were counted in both HOS and control. The number of sperm with curled tails in control were subtracted from that in HOS in order to obtain HOS-reactive/positive spermatozoa.

Acrosome integrity: Giemsa stain was used for assessment of acrosome integrity of spermatozoa. From the extended semen (10 µL), a smear was prepared and stained in 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 slide was air dried and examined under oil immersion (100×). About 200 sperms with intact acrosome and damaged acrosome (partially or totally) were counted in various fields and expressed in percentage.

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 and expressed in percentage.

Determination of lipid peroxidation (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 2 min 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: Statistical evaluations were done using SPSS (Version 26) program. The data on motility, live sperm count, plasma membrane integrity, acrosome integrity and *in vitro* capacitation/acrosome reaction and lipid peroxidation in different extenders at different hours of storage were analyzed by comparing the means through repeated measures of ANOVA. Proportions were normalized by the arcsin method. The results were

expressed in mean±SE. A confidence level of *P<0.05* was considered significant.

RESULTS AND DISCUSSION

In the extended semen, the results generated on percentage of different sperm attributes and lipid peroxidation at different time points have been presented in Tables 1-3.

Sperm motility: The mean proportion of motile sperm decreased progressively (*P<0.05*) from 0-120 h of preservation in all extenders (Table 1). Among the six extenders, BTS and Modena displayed higher (*P<0.05*) sperm motility as compared to GEPS from 48-120 h and in comparison to Safe Cell, ASP and MIII from 24-120 h. Likewise, GEPS also had higher (*P<0.05*) proportion of motile sperm from 24-120 h than all commercial diluents. The minimum 60% motility is essentially required in extended semen sample (Johnson *et al.* 2000). In the present study, this level was maintained until 72 h in BTS and Modena, 48 h in GEPS and up to 24 h in the remaining extenders. Similar studies (De Ambrogi *et al.* 2006, Mapeka *et al.* 2012, Govindasamy *et al.* 2016) with different extenders also showed higher motility with BTS and Modena as compared to the other extenders. High motility in Modena and BTS extenders could be due to their specific composition. De Ambrogi *et al.* (2006) demonstrated that the presence of bovine serum albumin (BSA) cysteine, Ethylenediaminetetraacetic acid (EDTA) and Tris hydroxymethyl aminomethane (TRIS) in Modena improves the semen storage capacity parameters, especially motility. Moreover, BTS and Modena extended semen also reduced variation among the boars, although the individual boar's performance always was the key to influence sperm motility between extenders.

Sperm viability: From the initial (0 h) period of storage until 120 h of preservation, a significant (*P<0.05*) reduction in the proportion of viable spermatozoa was observed in all the six selected extenders (Table 1). The mean live sperm percentages were higher (*P<0.05*) in BTS and Modena at 24-48 h (*P<0.05*) and at 72-120 h (*P<0.01*) than in remaining extenders. Intact and viable plasma membrane is also essential sperm characteristic which provide structural

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

Extender	Preservation period (h)					
	0	24	48	72	96	120
			Progressive sperm motility (%)			
Modena	80.6±1.3$^{a!@}$	72.6±1.8$^{b!}$	65.8±1.0$^{c!}$	60.8±1.5$^{d!}$	53.7±1.9$^{e!}$	44.7±0.7$^{f!}$
BTS	82.8±1.7$^{a!@}$	74.2±1.1$^{b!}$	68.4±1.7$^{c!}$	61.4±1.4$^{d!}$	55.3±2.2$^{e!}$	47.1±1.6$^{f!}$
GEPS	83.3±1.4$^{a!}$	72.7±1.6$^{b!}$	60.1±2.1$^{c@}$	50.1±1.3$^{d@}$	41.2±2.0$^{e@}$	33.8±0.9$^{f@}$
Safe Cell	79.4±1.7$^{a@\#}$	65.2±1.3b$^{@}$	48.9±1.8$^{c\#\wedge}$	37.8±1.4$^{d\#}$	28.3±1.6$^{e\#\wedge}$	21.9±1.1$^{f\#}$
Androstar Plus	79.7±1.4$^{a@\#}$	60.8±0.9$^{b\#}$	51.5±1.3$^{c\#}$	39.1±0.9$^{d\#}$	31.6±1.4$^{e\#}$	22.1±1.7$^{e\#}$
M III	77.6±1.5$^{a\#}$	59.9±1.2$^{b\#}$	45.7±1.6$^{e\wedge}$	40.2±1.1$^{d\#}$	26.8±1.0$^{e\wedge}$	19.3±0.8$^{f\#}$
			Sperm viability (%)			
Modena	92.7±2.2$^{a!}$	86.6±0.9$^{b!}$	75.3±1.6$^{c!}$	69.0±2.1$^{d!}$	61.9±1.4$^{e!}$	53.2±1.7$^{f!}$
BTS	89.4±1.3$^{a!@}$	84.8±2.3$^{b@}$	73.1±1.9$^{c!}$	66.7±1.6$^{d!}$	60.2±1.7$^{e!}$	51.6±1.6$^{f!}$
GEPS	86.3±1.8$^{a@}$	77.7±1.6$^{b\#}$	60.3±1.5$^{c@}$	49.8±1.0^{d+}	42.5±1.6^{e+}	31.1±0.9^{f+}
Safe Cell	90.7±1.1$^{a!}$	79.1±1.5$^{b@\#}$	59.7±1.8$^{c@}$	47.6±1.8^{d+}	43.6±0.7^{e+}	34.6±1.2^{f+}
Androstar Plus	87.1±1.9$^{a@}$	76.2±1.7$^{b\#}$	58.8±2.2$^{c@}$	50.1±1.3^{d+}	39.8±1.8^{e+}	30.2±0.8^{f+}
M III	89.7±1.6$^{a!}$	78.8±1.2$^{b\#}$	61.4±1.2$^{c@}$	49.3±0.8^{d+}	40.3±2.1^{e+}	33.4±1.0^{f+}
			HOST-positive spermatozoa (%)			
Modena	63.5±0.9^{a}	56.5±1.1^{b}	44.2±1.8^{c}	37.8±1.6^{d}	30.2±0.7$^{e@}$	26.3±0.9$^{f@}$
BTS	62.3±1.6^{a}	53.6±2.2^{b}	43.3±1.4^{c}	39.0±0.8^{d}	36.1±1.3$^{e!}$	32.9±1.2$^{f!}$
GEPS	64.1±2.1^{a}	57.3±0.7^{b}	41.6±1.9^{c}	37.9±1.5^{d}	26.4±1.9$^{e\#}$	20.1±1.5$^{f\#}$
Safe Cell	60.8±0.7^{a}	54.9±1.4^{b}	42.1±1.0^{c}	35.6±1.2^{d}	21.9±1.6$^{e\wedge}$	10.8±0.8^{f*}
Androstar Plus	64.5±1.5^{a}	56.6±1.7^{b}	45.3±1.9^{c}	38.0±1.2^{d}	24.4±1.5$^{e\#\wedge}$	12.6±1.0^{f*}
M III	62.7±1.4^{a}	55.4±2.0^{b}	41.9±1.5^{c}	34.4±0.9^{d}	18.8±1.2^{e*}	15.1±1.3$^{f\wedge}$
			Acrosome integrity (%)			
Modena	89.1±1.5^{a}	84.6±0.9^{b}	79.7±1.0$^{c!}$	74.6±0.7$^{d!}$	65.7±1.1$^{e!}$	60.8±0.9$^{f!}$
BTS	90.7±2.3^{a}	83.3±0.7^{b}	77.1±1.7$^{c!}$	73.2±0.9$^{d@}$	68.1±2.2$^{e@}$	62.3±1.4$^{f@}$
GEPS	88.2±0.9^{a}	82.5±1.4^{b}	68.7±2.1$^{c@}$	63.4±1.7$^{d\#}$	50.0±1.5$^{e\#}$	40.4±0.8$^{f\#}$
Safe Cell	90.3±1.0^{a}	83.9±1.6^{b}	70.2±1.6$^{c@}$	60.8±0.8$^{d\#}$	51.4±1.2$^{e\#}$	44.1±1.5$^{f\#}$
Androstar Plus	87.8±0.7^{a}	81.2±2.2^{b}	67.4±1.0$^{c@}$	63.7±1.0$^{d\#}$	48.5±1.6$^{e\#}$	42.7±1.3$^{f\#}$
M III	91.2±1.2^{a}	82.4±1.8^{b}	70.7±0.9$^{c@}$	61.2±1.4$^{d\#}$	51.8±0.9$^{e\#}$	41.6±0.7$^{f\#}$

^{a,b,c,d,e,f}, P<0.05; from corresponding values in a row. $^{!, @, \#, \wedge, *}$ P<0.05; from corresponding values in a column. ! vs + P<0.01; from corresponding values in a column. BTS, Beltsville Thawing Solution; GEPS, Glucose sodium salt of EDTA-Potassium sodium tartrate-sodium citrate dehydrate.

Table 2. Percentage of *in vitro* capacitation/acrosome reaction (Mean±SE) in liquid boar semen stored up to 120 h at 17°C in different extenders

Extender	Preservation period (h)					
	0	24	48	72	96	120
Modena	4.0±0.9$^{a!}$	4.9±0.7$^{a!}$	6.1±0.7$^{a!}$	10.6±0.6$^{b!}$	24.7±1.4$^{c!}$	31.3±1.2$^{d@}$
BTS	4.5±0.6$^{a!}$	4.7±0.9$^{a!}$	5.5±0.8$^{a!}$	11.7±0.8$^{b!}$	26.1±1.2$^{c!}$	37.5±1.1$^{d!}$
GEPS	3.9±0.7$^{a!}$	4.4±0.5$^{a!}$	5.3±0.5$^{a!}$	11.1±0.5$^{b!}$	17.3±0.9$^{c\#}$	22.6±1.4$^{d\#}$
Safe Cell	4.1±0.8$^{a!}$	4.3±0.7$^{a!}$	6.3±0.9$^{a!}$	9.9±1.0$^{b!}$	18.7±1.0$^{c@\#}$	24.7±1.6$^{d\#}$
Androstar Plus	5.1±0.6$^{a!}$	5.6±0.8$^{a!}$	6.0±0.7$^{a!}$	10.3±0.7$^{b!}$	15.9±1.1$^{c\#}$	22.9±0.8$^{d\#}$
M III	3.7±0.8$^{a!}$	3.9±0.9$^{a!}$	5.9±1.0$^{a!}$	9.7±0.9$^{b!}$	20.1±1.5$^{c@}$	25.0±1.1$^{d\#}$

^{a,b,c,d,e,f}, P<0.05; from corresponding values in a row. $^{!}$, $^{@,\#,\wedge,*}$ P<0.05; from corresponding values in a column. BTS, Beltsville Thawing Solution; GEPS, Glucose sodium salt of EDTA-Potassium sodium tartrate-sodium citrate dehydrate.

integrity and functional activity to spermatozoa and helps penetrate the barriers around ovum across species (Yi *et al.* 2004). Similar studies also depicted higher live sperm percentages than motility, possibly due to malfunctioning of cell mitochondria leading to loss of motility in spermatozoa, while they still had intact membranes (Frydrychová *et al.* 2010, Karageorgiou *et al.* 2016). From 24-120 h, BTS and Modena extenders exhibited higher live sperm percentages than in remaining ones. These findings are in accordance with the observations of a previous report by Govindasamy *et al.* (2016). Further, a drift of higher sperm viability in Modena than in BTS was noticed throughout storage. Modena contains cysteine which owing to its antioxidant properties plays an important role in maintenance of sperm membrane through inhibition of lipid peroxidation (LPO; Johnson *et al.* 2000). LPO has been regarded as the major cause of cell membrane impairment leading to decreased viability of spermatozoa (Kumaresan *et al.* 2009). Furthermore, as reported earlier, Modena contains BSA that plays a strategic role in decreasing LPO and subsequently reinforces the plasma membrane (De Ambrogi *et al.* 2006), thereby leading to higher proportion of viable membranes in the current study.

Hypo-osmotic swelling test and acrosome integrity: Irrespective of extender, there was reduction (*P<0.05*) in HOS-positive sperm population during preservation (Table 1). With respect to extenders, no difference (*P>0.05*) in the percentages of HOS-positive sperm was noticed until 72 h of storage period. At 96 h and 120 h of preservation, BTS maintained highest (*P<0.05*) proportion of HOS-positive sperm followed by Modena, GEPS, ASP, Safe Cell and M III. Compared to per cent sperm viability, the proportion of HOS-reactive spermatozoa responding by swelling were lower in the current study. These observations are in consonance with the findings of previous studies (Pérez-Llano *et al.* 2001) which described that this difference may partially be due to the fact that the membranes of few spermatozoa become damaged as they come in contact with hypo-osmotic solution, despite having motility. As observed in previous sperm attributes, the changes in mean percentages of intact acrosomes decreased (*P<0.05*) in all extenders with increased storage time intervals (Table 1). Between extenders, the per cent acrosome integrity was nearly similar (*P>0.05*) at 0 h and 24 h of preservation. From 48 h and until 120 h of storage, the number of spermatozoa showing intact acrosomes was higher (*P<0.05*) in BTS and Modena extenders in comparison to remaining ones. Johnson *et al.* (2000) demonstrated that abundance of unsaturated fatty acids and lack of antioxidant-rich cytoplasmic component contributes toward vulnerability of sperm membrane to LPO. Moreover, excessive production of ROS by spermatozoa has been linked to plasma membrane destruction during preservation (Kumaresan *et al.* 2009). In the current study, there was an apparent loss of sperm population with intact membranes and acrosomes at consecutive measurements up to 120 h of storage in all extenders.

In vitro capacitation/acrosome reaction: Irrespective of extenders, the percentages of *in vitro* capacitated/ acrosome-reacted spermatozoa exhibited a slow (*P>0.05*) increase from 0-48 h which became rapid (*P<0.05*) from 72 h of storage until 120 h in all extenders (Table 2). With respect to extenders, the *in vitro* capacitation/acrosome reaction was similar (*P>0.05*) in all six extenders until

Table 3. Lipid peroxidation (MDA, µmole/109 sperm) levels (Mean±SE) in liquid boar semen stored up to 120 h at 17°C in different extenders

Extender	Preservation period (h)					
	0	24	48	72	96	120
Modena	0.33±0.04$^{a!}$	0.44±0.03$^{b!}$	0.56±0.06$^{c!}$	0.73±0.08$^{d!}$	0.91±0.09$^{e!}$	1.22±0.14$^{f\wedge}$
BTS	0.35±0.03$^{a!}$	0.40±0.05$^{b!}$	0.61±0.03$^{c!}$	0.75±0.07$^{d!}$	0.89±0.05$^{e!}$	1.24±0.09$^{f\wedge}$
GEPS	0.31±0.07$^{a!}$	0.55±0.06$^{b@}$	0.72±0.05$^{c@}$	0.90±0.03$^{d@}$	1.12±0.08$^{e@}$	1.63±0.11$^{f@}$
Safe Cell	0.29±0.09$^{a!}$	0.59±0.04$^{b@}$	0.70±0.03$^{c@}$	0.87±0.04$^{d@}$	1.17±0.06$^{e@}$	1.56±0.07$^{f@}$
Androstar Plus	0.37±0.12$^{a!}$	0.51±0.03$^{b@}$	0.69±0.04$^{c@}$	0.92±0.07$^{d@}$	1.14±0.05$^{e@}$	1.48±0.12$^{f@}$
M III	0.28±0.04$^{a!}$	0.57±0.06$^{b@}$	0.72±0.06$^{c@}$	1.03±0.08$^{d@}$	1.26±0.10$^{e@}$	1.59±0.08$^{f@}$

^{a,b,c,d,e,f}, P<0.05; from corresponding values in a row. $^{!}$, $^{@,\#,\wedge,*}$ P<0.05; from corresponding values in a column. BTS, Beltsville Thawing Solution; GEPS, Glucose sodium salt of EDTA-Potassium sodium tartrate-sodium citrate dehydrate.

72 h of preservation. At 96 h, the proportion of acrosome-reacted spermatozoa were higher (*P<0.05*) in BTS and Modena than in other extenders. At 120 h of storage, BTS exhibited the highest (*P<0.05*) acrosome reaction among all extenders. Similarly, the semen extended in Modena also revealed a higher (*P<0.05*) percentage of *in vitro* acrosome-reacted spermatozoa than in the remaining four extenders. Nevertheless, the percentage of acrosome reaction was similar (*P>0.05*) in GEPS, Safe Cell, ASP and M III at 120 h of storage period. Comparative studies showing the efficacy of selected extenders on *in vitro* capacitation/acrosome reaction during liquid preservation of boar semen are not available in literature. Bicarbonates present in the BTS extender stimulate calcium ion influx, rapid and reversible organizational modifications in sperm membrane, activation of sperm surface proteins thereby binding intact acrosome cells to zona pellucida and consequent acrosome reaction (Conejo-Nava *et al.* 2003). Therefore, presence of sodium hydrogen carbonate in BTS extender might have led to better capacitation status following preservation for up to 120 h in the present study.

Lipid peroxidation (LPO): The mean levels of LPO as manifested by malondialdhyde (MDA) produced are presented in Table 3. At 0 h, the MDA concentrations were low which increased (*P<0.05*) up to 120 h of preservation in all the extenders. From 24-120 h of storage, the MDA concentrations were lower (*P<0.05*) in semen preserved in BTS and Modena in comparison to other extenders. Previous studies (Kumaresan *et al.* 2009, Karunakaran *et al.* 2017, Menegat *et al.* 2017) using different extenders (BTS, Safe Cell and ASP) also revealed that MDA concentrations increased considerably as the preservation progressed. In the present study, MDA levels at 120 h of storage exhibited three to five-fold increase in different extenders in comparison to native semen. Alternatively, there was 25-50% reduction in sperm motility at 120 h of preservation in different diluents compared to fresh sperm motility. Similar decline in per cent sperm viability, plasma membrane integrity and acrosome integrity were observed as the storage period advanced. The decreased sperm attributes during storage in the present study may be related to increased concentrations of MDA. These findings are in agreement with observations of Kumaresen *et al.* (2009) who showed that increased MDA levels adversely affect sperm functions. The generation of ROS triggers the LPO cascade leading to loss of membrane integrity, decreased cell function, reduced total antioxidant capacity and ultimately sperm death (Bucak *et al.* 2010). Thus, it is probable that ROS-induced sperm destruction occurs in higher percentage during storage. Eventually, the lower increment of MDA in Modena could be due to the scavenging action of cysteine on free radicals.

In conclusion, liquid storage of boar semen at 17°C in Beltsville Thawing solution (BTS) and Modena extenders were more effective in providing better sperm attributes and reduced oxidative stress as compared to other extenders.

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