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Efficacy of different extenders on sperm characteristics and fertility in crossbred pigs of north-eastern India

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ABSTRACT

The study was conducted to deduce the efficacy and suitability of three extenders: Beltsville thawing solution (BTS), Kiev and Modena extenders, on the preservation and fertility of boar semen. Semen ejaculates with more than 70% sperm motility from six Hampshire boars were used and preserved at 17 °C. The extended semen was evaluated for motility, viability, acrosomal and plasma membrane integrity from day 0 to 5 of preservation. There was a significant ($P<0.01$) reduction in sperm motility and viability between days 0 to 4, but no significant difference between day 0 and 2. BTS and Modena maintained significantly ($P<0.05$) higher sperm motility and viability as compared to Kiev. Plasma membrane integrity did not differ significantly up to day 2, but was significantly reduced on days 3 ($P<0.05$) and 4 ($P<0.01$). BTS and Modena extenders had significantly ($P<0.05$) higher plasma membrane integrity. By day 5, the percentage of mean sperm with intact acrosome was 80.4 ± 2.5, 81.2 ± 2.3 and 75.7 ± 2.7, in BTS, Modena and Kiev, respectively, with significant ($P<0.05$) increases in acrosomal damage after two days of storage, irrespective of all extenders. Farrowing rate and litter size did not differ significantly during the initial period of storage, but there was a significant difference after day 3 ($P<0.05$) in all extenders used. BTS and Modena maintained a farrowing rate of more than 60% on day 5, in contrast to 50% with the Kiev extender. The study concluded that both BTS and Modena are better extenders for short term storage of boar liquid semen as compared to the Kiev extender, and could be used efficiently in crossbreeding of pigs of north-eastern India.

Key words: extenders, liquid boar semen, sperm motility, membrane integrity, farrowing rate, litter size

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Introduction

Artificial insemination (AI) is an important technique, contributing to the improvement of livestock genetic potential through rapid dissemination of superior germplasm and faster genetic gain. The use of AI in the swine industry is not exception particularly liquid semen preserved at 15-20 °C (JOHNSON et al., 2000). Liquid semen is the preferred method of boar semen storage as compared to frozen semen, which has low semen dose output, in addition to decreased sperm survival with low fecundity (GILLMORE et al., 1998). In contrast, liquid semen has the advantage of maintaining fertility, even with low sperm count, superior storage competence (CLARKE and JOHNSON, 1987) in addition to improved reproductive efficiency, as well as economic profit in the swine industry (WATERHOUSE et al., 2004). However, the major drawback of boar liquid semen, shown in several studies, is the decline in fertility during storage periods, in relation to the type of extenders used (WATERHOUSE et al., 2004; DE AMBROGI et al., 2006; FRYDRYCHOVÁ et al., 2010). A variety of extenders, whether short term (Beltsville Thawing Solution (BTS), Illinois Variable Temperature (IVT), and Vital) or long term, (Modena, Kiev, Androhep, Acromax and Zorlesco) have been used for the extension of boar semen with varied results in different swine breeds (GADEA, 2003; VYT et al., 2004; DUBE et al., 2004; MAPEKA et al., 2012). Although short-term extenders are widely used, the demand for use of long-term extenders is on the rise due to their better and longer preservation qualities for boar semen (FRYDRYCHOVÁ et al., 2010). These extenders protect the sperm against reactive oxygen species, cold shock, and also act as an energy source providing a buffering effect against sperm cell waste, and they prevent growth of microbes. Through testing the efficiency of different extenders for semen storage, in *in vitro* assays of for example motility, membrane integrity and acrosome status, they have been widely proven to be reliable (AMANN, 1989). Furthermore, since it has been shown that the success of fertilization is influenced by both *in vitro* and *in vivo* sperm physiological changes, hence the *in vivo* fertilization rate is also an important parameter to be considered for deducing the effect of extenders during boar liquid semen preservation (SOEDE et al., 1995).

AI is considered to be an effective tool in crossbreeding programs of nondescript local pigs with superior germplasm in developing countries including India (VISALVETHAYA et al., 2010; KADIRVEL et al., 2013). The use of extenders in boar semen preservation has been shown, along with their effect on *in vitro* seminal parameters (KHAN et al., 2006), but there are no reports of the efficacy of extenders used for boar semen regarding the fertility outcome in crossbred sows (Hampshire × indigenous). This is yet to be studied. The present study was designed to evaluate the efficacy of extenders on boar sperm characteristics and fertility in the crossbred pigs of north-eastern India.

Materials and methods

Location of the study. The present study was carried out at the Animal Production Division, ICAR Research Complex for the North East Hill Region, Meghalaya, India. The study site is located at a height of 1010 m above mean sea level, with annual rainfall ranging from 2239 to 2953 mm. The annual maximum and minimum temperatures range between 21.1 to 29.2 °C and 7 to 20.9 °C, respectively. The present study was carried out with the approval of the institute's animal ethics committee.

Animals, semen collection, processing and evaluation. Six Hampshire boars (age: 2-4 years) routinely used for breeding programs on the pig breeding farm were utilized for the present study. The breeding boars were maintained under uniform management conditions, and fed with a balanced concentrate feed twice daily. The boars were trained for semen collection by gloved hand technique with a dummy sow (IMV Technologies, France). A total of 72 semen ejaculates, 12 ejaculates from each boar, were collected twice weekly. Semen was collected in a sterilized plastic bottle, and the gel fraction was strained using a Buchner funnel with gauze. The gel free semen samples were used for further processing and preservation. Immediately after collection, the semen volume, color, consistency, pH and motility were evaluated, and those ejaculates having more than 70% sperm motility were further utilized for the study. Out of 72 ejaculates, 6 ejaculates were rejected and 66 samples were suitable for further processing and preservation. The concentration of spermatozoa (millions per milliliter) in the semen was determined by the hemocytometer method, adopting the red blood cell counting procedure (SALISBURY et al., 1985).

Semen dilution and preservation. Beltsville Thawing Solution (PURSEL and JOHNSON, 1975), Modena (MORETTI, 1981), and Kiev (PLISKO, 1965) were used in the study. Each ejaculate was divided into three parts and extended at the rate of 1:3 to 1:4 depending upon the concentration of each of the extenders under study. Approximately 90 mL of the extended semen containing ~3 billion sperm was packed in a sachet using a filling and sealing machine (IMV Technologies, France), and preserved at 17 °C in a biochemical oxygen demand incubator (Narang Scientific Works, India). The efficacy of the three extenders, and the duration of preservation of the extended semen in the extenders were evaluated regarding sperm motility, viability, acrosomal and membrane integrity, along with fertility from days 0 to 5.

Assessment of sperm motility and viability. A drop of preserved semen was kept on a clean, pre-warmed glass slide (37 °C) and after covering it with a cover slip, it was examined under high power magnification (40×) using a phase contrast microscope (Olympus-BX51, Tokyo, Japan). The percentage of spermatozoa with normal, vigorous, and forward linear motion was subjectively assessed to the nearest 5% in five different areas of the sample on each slide. Sperm viability was assessed by the eosin-nigrosin staining technique (CAMPBELL et al., 1953). On each slide, 200 spermatozoa were

examined under an oil immersion (100×) microscope (Olympus-BX51, Tokyo, Japan). Both fully and partially stained spermatozoa were counted as dead.

Assessment of acrosomal integrity. Sperm acrosomal integrity was determined in the extended semen by the Giemsa staining method (WATSON, 1975), with modifications to reduce the duration of staining. A thin smear was prepared from the extended semen and air dried. The semen smear was fixed in Hancock's fixative for 15 min, washed in tap water and finally rinsed with distilled water. Then the smear was stained with Giemsa stain containing 3 mL of Giemsa stock solution, diluted with 2 mL of Sorenson's phosphate buffer (0.133 M Na_2HPO_4 and 0.133 M KH_2PO_4, pH 7.2) and 35 mL of distilled water for two hours at 36 °C in a staining jar. The excess Giemsa stain was then removed by rinsing the smeared slides in distilled water, they were air dried and mounted in the DPX mountant. The slide was examined under the oil immersion microscope (100×) for acrosomal integrity. The acrosomes manifesting marked swelling, knobbing, ruffling or incomplete contour, denudation (lost) and others (including knobbed) were considered as damaged, and the remaining acrosomes considered as intact acrosome. A total of 200 spermatozoa were counted in a duplicate slide for each semen sample.

Assessment of membrane integrity. Sperm membrane integrity was assessed by the carboxyfluoresceindiacetate (CFDA)/propidium iodide (PI) fluorescent staining technique (HARRISON and VICKERS, 1990). For this study, a stock solution of CFDA and PI was prepared at the concentration of 1 mg/mL in Dimethyl sulfoxide (DMSO) and 1 mg/mL in Phosphate Buffer Saline (PBS), respectively. The stock CFDA was added at a final concentration of 20 µM to 0.5 mL of sperm suspension. After 5 min of incubation, PI was added at a final concentration of 15 µM to the sperm suspension. The stained sperm suspension was covered with a coverslip and observed under a fluorescent microscope (Olympus-BX51, Tokyo, Japan). Each sample was assessed twice, and at least 200 spermatozoa per slide were classified as: intact membrane (CFDA staining; green fluoresces,), dead (PI staining; red fluoresces) or morbid spermatozoa (both green and red fluoresces).

Assessment of fertility. To assess the farrowing rate and litter size, crossbred (Hampshire × indigenous) sows, maintained under standard management conditions on the pig breeding farm of the institute, were used. Estrus detection was carried out twice daily and the sows exhibiting standing estrus were inseminated with a golden pig catheter (IMV Technologies, France). A total of 139, 135 and 134 sows were inseminated with three billion sperm in 80 mL of semen preserved in BTS, Modena and Kiev extenders, respectively. The inseminated, non-cycling sow/gilts were diagnosed for pregnancy after 6 weeks by the Doppler method using a trans-abdominal probe. After confirmation of pregnancy, pregnant sow/gilts were separated from non-pregnant sows and kept in individual pens. The farrowing rate (the ratio of the number of sows that farrowed to the

number sows inseminated) and litter size at birth (total number of piglets born per farrow) were calculated after farrowing.

Statistical analyses. The data on sperm motility, viability, membrane integrity and fertility in the different extenders on different days of storage were analyzed by comparing the means through multiple ANOVA (LSD and Duncan multiple range test), SPSS version (13.0). Data were expressed in mean ± SE. Analysis of variance between the extenders and comparison of means at 5% significance level by Duncan's multiple range test (DMRT) was carried out. Results were considered significant at $P<0.05$.

Results

Sperm motility and viability. The percentages of sperm motility and viability in BTS, Modena and Kiev, on different days of preservation, are presented in Fig. 1 and 2. A general trend of liner decline in motility and viability of spermatozoa was observed in all three extenders with increasing days of preservation. There was a significant ($P<0.01$) reduction in sperm motility and viability from day 0 to 4 irrespective of extender, but it did not differ significantly between day 0 and 2. With the advance in storage days there was a significant reduction in sperm motility and viability by days 3 ($P<0.05$) and 4 ($P<0.01$). With respect to extenders, Modena and BTS maintained significantly higher ($P<0.05$) sperm motility and viability until days 4 and 5, respectively, as compared to Kiev.

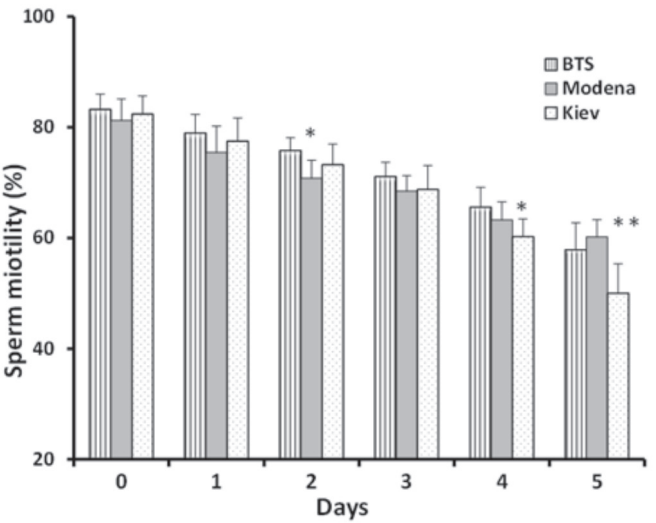

Fig. 1. Sperm motility (%) of liquid boar semen preserved in different extenders (*P<0.05, **P<0.01) differs significantly

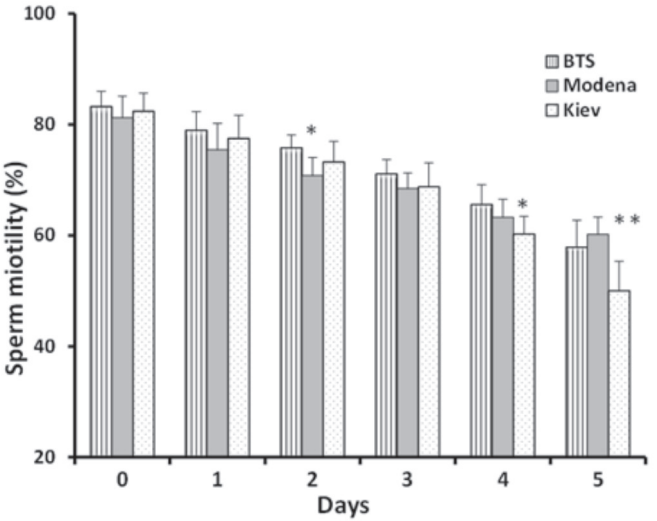

Fig. 2. Percentage of live sperm in liquid boar semen preserved in different extenders (*P<0.05, ** P<0.01) differs significantly

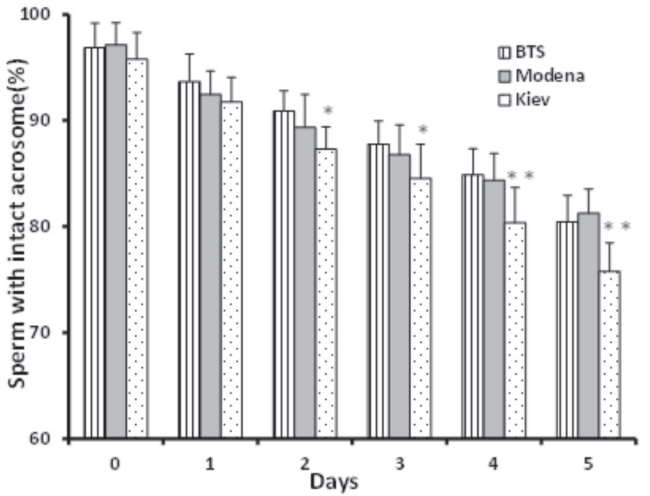

Fig. 3. Percentage of sperm with intact acrosomes in liquid boar semen preserved in different extenders (*P<0.05, ** P<0.01) differs significantly

Acrosomal integrity. The percentage of spermatozoa with intact acrosomes on day 0 was 96.87 ± 2.34%, 97.12 ± 2.14% and 95.78 ± 2.53% in BTS, Modena and Kiev extenders, respectively, showing no significant difference in the percentage of intact acrosome between or within the diluents (Fig. 3). Although the percentage of sperm population with acrosomal damage was significantly ($P<0.05$) increased after two days of storage in all extenders, the number of sperms with intact acrosomes was significantly ($P<0.05$) higher in BTS and Modena than in Kiev extender from day 2 of preservation (Fig. 3).

Table 1. Sperm plasma membrane integrity of preserved liquid boar semen in different extenders

Days	Extenders								
	BTS			Modena			Kiev		
	Intact membrane (%)	Dead (%)	Morbid (%)	Intact membrane (%)	Dead	Morbid	Intact membrane (%)	Dead	Morbid
0	87.42 ± 1.34[a]	9.23 ± 0.76[a]	4.45 ± 0.17[a]	86.73 ± 1.44[a]	10.23 ± 0.67[a]	4.63 ± 0.23[a]	86.89 ± 1.57[a]	9.76 ± 0.86[a]	4.78 ± 0.47[a]
1	83.67 ± 2.34[a]	11.56 ± 0.96[a]	6.56 ± 0.69[a]	78.64 ± 2.64[b]	12.54 ± 1.17[a]	7.56 ± 0.74[a]	82.67 ± 2.64[a]	10.87 ± 1.23[a]	6.34 ± 0.79[a]
2	77.45 ± 3.56[b]	14.34 ± 1.37[b]	8.89 ± 0.93[b]	74.37 ± 3.1[b]	15.86 ± 1.64[b]	9.76 ± 1.43[b]	73.91 ± 3.87[b]	15.36 ± 1.36[c]	9.81 ± 1.32[b]
3	73.76 ± 4.45[b]	17.56 ± 1.63[b]	11.97 ± 1.48[b]	71.43 ± 3.87[c]	18.66 ± 2.01[b]	12.76 ± 1.65[b]	68.04 ± 2.87[c]	19.12 ± 2.13[c]	12.43 ± 1.38[b]
4	66.45 ± 5.87[c]	21.23 ± 2.33[c]	13.56 ± 1.38[c]	68.76 ± 2.27[c]	22.17 ± 2.18[c]	13.87 ± 1.18[c]	61.45 ± 3.37[c]	23.43 ± 2.83[d]	15.46 ± 1.17[c]
5	58.27 ± 4.35[c]	26.13 ± 1.61[c]	16.65 ± 1.21[c]	61.23 ± 2.34[c]	23.42 ± 1.75[c]	15.34 ± 1.67[c]	54.17 ± 2.78[d]	27.56 ± 2.46[d]	18.87 ± 1.45[d]

Data shown as Mean ± SE, Values with different superscript in the column differ significantly ($P\leq0.05$)

Membrane integrity. Three populations of cells are discriminated by fluorescence microscopy when staining sperm with CFDA/PI: a) live spermatozoa stain green due to retention of the hydrolyzed products of CFDA; b) sperm cells exhibit green acrosome and red nuclei, indicating damaged plasma membrane, but an intact acrosome and c) sperm with red nuclei depicting dead or degenerated cells. The mean percentages of these three populations in the different extenders on different days is shown in Table 1. Although the sperm population with intact membranes did not differ significantly until day 1 in all

extenders, there was a significant reduction on day 4 ($P<0.01$). Although the number of dead spermatozoa significantly ($P<0.01$) increased progressively during storage from day 0 to 4 in all extenders, BTS and Modena extenders had a significantly ($P<0.05$) higher percentage of sperm population with intact membranes through days 3 and 4 as compared to the Kiev extender group.

Fertility rate. The farrowing rate, average litter size and number of live born per litter, following AI with semen preserved in BTS, Modena and Kiev on different days of preservation are shown in Table 2. There was no significant difference between days regarding farrowing rate and litter size from day 0 to day 1 using these different extenders. However, the farrowing rate was significantly reduced after day 3 ($P<0.05$) and day 4 ($P<0.01$) in all extenders. Similarly, the litter size was also significantly reduced by day 4 in all the extenders. However, the farrowing rate and litter size did not differ between BTS and Modena on different days of preservation.

Table 2. Farrowing rate and litter size with preserved liquid boar semen in different extenders

Days	Extenders					
	BTS		Modena		Kiev	
	Farrowing rate (%)	Litter size at birth	Farrowing rate (%)	Litter size at birth	Farrowing rate (%)	Litter size at birth
0	82.26^{a}	9.46 ± 0.6^{a}	79.16^{a}	9.12 ± 0.6^{a}	81.80^{a}	9.67 ± 0.3^{a}
1	80.00^{a}	9.61 ± 0.5^{a}	77.20^{a}	8.92 ± 0.4^{a}	78.26^{a}	8.95 ± 0.4^{a}
2	76.40^{a}	8.70 ± 0.3^{a}	76.00^{b}	8.74 ± 0.7^{a}	76.19^{b}	9.10 ± 0.5^{a}
3	77.20^{a}	8.68 ± 0.8^{a}	72.72^{b}	8.35 ± 0.9^{b}	68.18^{b}	8.34 ± 0.4^{b}
4	69.56^{b}	8.18 ± 0.7^{b}	71.14^{b}	7.52 ± 0.7^{b}	54.16^{c}	7.13 ± 0.62^{c}
5	61.90^{c}	7.98 ± 0.5^{c}	61.90^{c}	7.67 ± 0.6^{b}	50.00^{d}	7.23 ± 0.65^{c}

Data shown as Mean ± SE, Values with different superscript in the column differ significantly ($P<0.05$).

Discussion

In the present study, the effect of BTS, Kiev and Modena extenders on boar sperm characteristics and *in vivo* fertility performance was studied in local crossbred pigs in north-eastern India. Considering sperm motility, a crucial feature for passage through the cervix, utero-tubal junction and, even more important, through the cumulus, the present study revealed that BTS and Modena maintained significantly ($P<0.05$) higher sperm motility and viability as compared to the Kiev extender. This result is in agreement with the earlier reports by KOMMISRUD et al. (2002), LALRINTLUANGA et al. (2002) and MAPEKA et al. (2012). However our results are contrary to SHIMATSU et al. (2002), who reported that the Modena extender was more efficient than Kiev and BTS for semen

storage for 5-7 days at 15 °C. The low sperm motility and viability in the Kiev extender group may be attributed to its higher osmotic pressure (420 mosM) as compared to the other extenders (BTS: 330 mosM; Modena: 282 mosM) used in the present study (GADEA, 2003). Although boar sperm can tolerate a wide range of osmolality (240 to 380 mosM), it has been shown that isotonic or hypotonic media offer better preservation and fertilizing capacity as compared to hypertonic media and extenders (WEITZE, 1990). In contrast, the long-term extender, Modena, increases the semen storage capacity parameters owing to its composition of Tris (hydroxymethyl) aminomethane (TRIS), Bovine serum albumin (BSA), Ethylenediaminetetraacetic acid (EDTA) and cysteine. TRIS acts as a pH regulator, whereas BSA protects sperm against cold shock (DE AMBROGI et al., 2006) and strengthens the sperm plasma membrane by reducing lipid peroxidation (DIXON and KREIDER, 1981). EDTA is present in the both BTS and Modena, as a chelating agent that blocks the action of calcium as a mediator of sperm capacitation and acrosome reaction. Furthermore, EDTA inhibits or decreases aldolase enzyme action, and thus may help in preserving sperm motility for a longer period (PLISKO, 1965). EDTA also counteracts toxicity by chelating metal ions in the semen. In addition the cysteine in the Modena extender acts as an antioxidant, to stabilize sperm plasma membranes and inhibit sperm capacitation (JOHNSON et al., 2000). Although there was no significant difference in the sperm motility and viability between BTS and Modena, BTS maintained a trend of non-significantly higher sperm motility, viability and membrane integrity through days 0 to 4, and Modena until day 5 of semen preservation, as reported earlier by DE AMBROGI et al., (2006). The increase in sperm motility and viability in BTS and Kiev extenders may be attributed to the difference in the composition of the extenders, contributing to the preservation of semen, since BTS contains potassium chloride which helps in the maintenance of the sodium potassium pump and prevents intracellular potassium depletion, thereby facilitating sperm motility (ALVAREZ and STOREY, 1982).

Plasma and acrosome morphology, with its functional integrity, are of fundamental importance, not only in attachment, species specific binding and zona penetration, but also for fertility across species (SCHILL et al., 1988; YI et al., 2004). In the present study, there was no significant difference between the extenders in the acrosomal integrity of spermatozoa from day 0 to 1, but with advancement of days there was a significant difference in plasma and acrosomal integrity between the extenders, as reported in earlier studies (PURSEL and JOHNSON 1975; LALRINTLUANGA et al., 2002), due to the fact that there was a decrease in membrane fluidity during storage, especially at the sperm head region as compared to other regions, leading to possible capacitation-like changes thereby initiating the acrosome reaction. From earlier studies it has been well established that, with the decline in temperature, there is an inevitable reduction in the proportion of sperm that maintain normal membrane integrity, ultrastructure, and biochemical components (JOHNSON et al., 2000). For these reasons, in the present study there was a lower percentage of sperm

with intact plasma membranes with the advancement of storage days in all extenders, as reported earlier (FRYDRYCHOVÁ et al., 2010). However, BTS and Modena extenders had significantly ($P<0.05$) higher percentage sperm populations with intact membranes on day 3 and 4, when compared to the Kiev extender group. Furthermore, the higher percentage of sperm with intact plasma membranes and acrosomes in BTS in the study might be due to the presence of isotonic or hypotonic extenders, providing a protective role for plasma and acrosome membranes, as discussed earlier (GADEA, 2003; VYT et al., 2004). Although the influence of the boar on membrane and acrosomal integrity has been documented earlier, this study fails to ascertain such associations (KOMMISRUD et al., 2002).

In the present study, a significantly higher farrowing rate and litter size in BTS and Modena was observed as compared to Kiev semen extender, from day 0 to day 5 of semen preservation. Seminal parameters, farrowing rate and litter size were not significantly different between day 0 and day 1 for different extenders. However, the farrowing rate was significantly reduced after day 3 ($P<0.05$) and day 4 ($P<0.01$) irrespective of the extender. Similarly, the litter size was also significantly reduced on day 3 and day 4 in all extenders. However, farrowing rate and litter size did not differ between BTS and Modena on different days of preservation in the present study. The farrowing rate and average litter size with BTS recorded in the present study were in accordance with TONIOLLI et al. (1998). Similarly, JOHNSON et al. (1988) reported higher farrowing rates and litter size in sows inseminated with BTS extended semen as compared to Modena. Hence, from this study it may be concluded that short term preservation with BTS was found to be more efficient than Kiev in terms of fertility, as reported earlier (BLICHFELDT et al., 1988).

Furthermore, the significant reduction of fertility rate and litter size with the advancement of storage days observed in the present study was in accordance with the earlier study by ALEXOPOULOS et al. (1996). Similarly, JOHNSON et al. (1988) reported that the farrowing rate for semen used on initial days of preservation after collection were superior to semen used after 3 days However, SHIMATSU et al. (2002) reported higher farrowing and litter size rates after insemination with extended miniature boar semen in Modena for 5-7 days. This variation might be due to difference in boar breed, management and the environmental conditions under which the study was conducted. On comparison between short-term and long term diluent, Modena revealed a non-significant difference in seminal as well as fertility parameters during the initial period of storage, but a significant difference ($P<0.05$) was observed thereafter and they were comparatively higher than in long-term extenders (Table 5 and 6). This reduction in farrowing rate and litter size with the advancement of storage, irrespective of extenders, may be attributed to the disruption in the sperm membrane integrity which initiates the destabilization process, thereby affecting sperm responsiveness to female signals, including cell death. Likewise,

farrowing rate and litter size also depend on the success of AI, which depends on factors including intensity and duration of estrus, timing of insemination, along with the extenders used in preserving the boar semen. This signifys that the choice of diluent should always be aimed at optimizing subsequent fertility rates and litter size in pig breeding (GADEA, 2003). From the present study it may be concluded that BTS and Modena extenders are more efficient than Kiev in terms of preservation of sperm characteristics and fertility at 17 °C and may be used effectively in crossbreeding native pigs of north-eastern India

Conflict of Interests

Authors declare there is no conflict of interest in this research work.

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SAŽETAK

Istraživanje je provedeno kako bi se utvrdila učinkovitost i prikladnost beltsvilskog (BTS), kijevskog i modenskog razrjeđivača za očuvanje i plodnost nerastova sjemena. Upotrijebljeni su ejakulati sjemena od 6 hempširskih nerastova s pokretljivošću spermija većom od 70%, koji su sačuvani na 17 °C. Razrijeđeno sjeme procjenjivano je od 0. do 5. dana s obzirom na pokretljivost, živost te cjelovitost akrosomalne i plazmine membrane. Tijekom razdoblja od 0. do 4. dana došlo je do signifikantnog (P<0,01) sniženja pokretljivosti i živosti spermija, no signifikantne razlike između nultog i drugog dana nisu utvrđene. U usporedbi s kijevskim razrjeđivačem, BTS i modenski razrjeđivači su održavali signifikantno (P<0,05) višu pokretljivost i životnost spermija. Cjelovitost plazmine membrane nije se signifikantno razlikovala do 2. dana, ali je signifikantno snižena tijekom 3. dana (P<0,05) i 4. dana (P<0,01). Cjelovitost plazmine membrane bila je signifikantno veća (P<0,05) pri uporabi BTS i modenskog razrjeđivača. Do 5. dana, prosječni postotak spermija s nepromijenjenim akrosomom iznosio je kod BTS razrjeđivača 80,4 ± 2,5, kod modenskog razrjeđivača 81,2 ± 2,3, a kod kijevskog razrjeđivača 75,7 ± 2,7. Pri tome je, bez obzira na razrjeđivač, od 2. dana utvrđen signifikantni porast (P<0,05)

spermija s oštećenim akrosomima. U početnom razdoblju stopa prasenja i veličina legla nisu se signifikantno razlikovale, no nakon 3. dana kod svih upotrijebljenih razrjeđivača razlike su bile signifikantne ($P<0,05$). Peti dan stopa prasenja kod BTS i modenskog razrjeđivača održala se preko 60% za razliku od kijevskog razrjeđivača kod kojeg je stopa prasivosti iznosila 50%. Istraživanjem se zaključuje da BTS i modenski razrjeđivači mogu, u odnosu na kijevski razrjeđivač, bolje poslužiti za kratkotrajno pohranjivanje nerastovog tekućeg sjemena, te kao takvi biti i učinkovitiji u križanjima svinja sjevernoistočne Indije.

Ključne riječi: razrjeđivači, nerastovo tekuće sjeme, pokretljivost spermija, cjelovitost membrane, stopa prasenja, veličina legla