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Comparative efficacy of three extenders on quality of boar semen during preservation at 15°C

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ABSTRACT

The present study was designed to compare the efficacy of 3 different extenders for preservation of boar semen at 15°C. Ejaculates (32) were evaluated and extended (1:4) in TRIXcell+, Modena and GEPS extenders and kept for 4 h of holding at 22°C. The samples were preserved in a BOD incubator at 15°C up to 168 h and evaluated for sperm motility, intact acrosome and HOST-reacted sperm at 0, 72, 96, 120, 144 and 168 h of preservation. The mean percentage of sperm motility, intact acrosome and HOST-reacted sperm was significantly higher in TRIXcell+ extender than Modena and GEPS at different hours of preservation. Out of the 3 extenders studied, TRIXcell+ was found to be the best followed by Modena and GEPS extenders respectively based on sperm motility, intact acrosome and HOST.

Keywords: Boar, Extender, Fertility, GEPS, Modena, Preservation, Semen quality, TRIXcell+

Artificial insemination (AI) contributes to the livestock genetic improvement through rapid dissemination of superior germplasm. Thus, it could be beneficial for meeting the demand of improved pig germplasm and help in faster propagation of elite germplasm. In the swine industry, liquid semen is the preferred method of boar semen storage (Weitze 1991) than frozen semen due to low semen dose output, in addition to decreased sperm survival with low fecundity (Gilmore *et al*. 1998).

A multitude of porcine semen extenders have been developed during the last few decades. Through the test of time, a list of extenders has dwindled to select a few that have proven worth to the global swine AI industry (Althouse *et al.* 1998). These extenders are used to create multiple insemination doses from a single ejaculate and contain buffers and nutrients that provide spermatozoa with an environment that maintains viability for three or more days post collection. The shelf life of extended semen has become increasingly important for flexible use of AI (Waberski *et al.* 2008) since the fertility of the diluted semen doses usually decline as their preservation period increases and the viability of preserved boar semen is strongly influenced by the composition of the extender used (Bresciani *et al.* 2013).

The major drawback of boar semen, shown in several studies, is the decline in fertility during storage periods, in relation to the type of extenders used (Frydrychova *et al.* 2010). Many of the extenders generally used has a storage period for up to 3–5 days but extenders having preservation capacity for longer periods have certain advantages which include possibility of long distance transport, conducting diagnostic tests on semen before use such as polymerase chain reaction (PCR) to detect presence of several viral antigen or a full analysis of semen quality, improving the organization of tasks at semen collection centres and to distribute the semen samples to several farms (Gadea 2003). Consequently, preservation of the fertilizing capacity of boar semen for several days remains a major target for the swine AI industry and also remains as a major challenge.

Success of AI depends on use of a suitable extender that maintains the quality of semen during prolonged preservation. Therefore, the present study was undertaken to investigate the efficacy of TRIXcell+, Modena and GEPS extenders on the quality of crossbred Hampshire boar semen during preservation at 15°C for 7 days.

MATERIALS AND METHODS

Four clinically healthy crossbred Hampshire breeding boars maintained at All India Coordinated Research Project (AICRP) on Pig, College of Veterinary Science, Guwahati, were selected. Ejaculates (32), 8 from each of the 4 boars were used. Semen was collected from each boar once weekly by simple fist method using a portable iron dummy as mount between January and May 2018. Immediately after collection, the semen was evaluated for different semen characteristics and ejaculates having more than 70% initial motility were selected for further processing and preservation. Each seminal ejaculate was split into 3

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fractions and extended (1:4) with TRIXcell+ (IMV Technologies, L'Aiglecedex, France), Modena and Glucose-sodium salt of EDTA-potassium sodium tartrate-sodium citrate dehydrate (GEPS) extenders and kept for holding at 22°C for 4 h. Thereafter samples were preserved in different glass beakers at 15°C in a BOD incubator up to 7 days (168 h). Prior to evaluation, the preserved semen samples were kept at 37°C for 2 min and gently shaken for homogenization; then evaluated for sperm motility, intact acrosome (Watson 1975) and HOST-reacted sperm (Revell and Mrode 1994) at 0 (immediately after extension), 72, 96, 120, 144 and 168 h of preservation. Females (40) maintained at AICRP on Pig, College of Veterinary Science and in and around Guwahati, Assam, India by private breeders were enrolled for AI. Semen extended (1:4) in the best extender (TRIXcell+) was packed in GTB bags (IMV Technologies, L'Aiglecedex, France) keeping 80 ml per dose. For AI, the same preserved semen held at 15°C in BOD incubator was utilized up to 120 h of preservation with simultaneous semen evaluation. Goldenpig Cathethers (IMV Technologies, L'Aiglecedex, France) were used to inseminate the estrus females with liquid semen of 0, 24, 48, 72, 96 and 120 h of preservation. The data were analyzed with one way ANOVA using the Statistical Analysis Systems (enterprise Guide 4.2 version) and Duncan's Multiple Range Test was applied to compare the differences between mean values.

RESULTS AND DISCUSSION

The mean values and analysis of variance of semen quality in different extenders for different hours of preservation at 15°C are presented in Table 1. Analysis of variance revealed that the sperm motility, intact acrosome and HOST-reacted sperm differed significantly (P>0.01) among different extenders and preservation periods.

The mean percentage of sperm motility differed significantly (P>0.05) among TRIXcell+, Modena and GEPS extenders at 0, 72, 96, 120, 144 and 168 h of preservation. However, it was significantly higher (P<0.05) in TRIXcell+ extender as compared to Modena and GEPS extenders at all hours of preservation. The mean percentage of sperm motility at each hour of preservation in Modena was significantly higher (P<0.05) than GEPS extender. Similar findings were also obtained by Bania (2017), van den Berg *et al*. (2014) and Saikia *et al*. (2016) who recorded significantly lower sperm motility in TRIXcell+, Modena and GEPS extenders. The significantly higher sperm motility in TRIXcell+ extender is unknown as its composition is a commercial secret. Moreover, significant higher sperm motility in Modena than GEPS could be attributed to the presence of Tris in Modena extender which are complex buffer agents that can regulate the pH over a wider range and did not depend on the temperature (Gadea 2003) and thus provided better buffering capacity. Significant decrease in the sperm motility in different extenders with increase in hours of preservation could be due to the progressive decline in nutrient content in the extenders with increased period of preservation. Excessive ROS formation by spermatozoa during preservation has also been associated with damaging plasma membrane and consequently reducing sperm motility (Kumaresan *et al.* 2009).

The mean percentage of intact acrosome was significantly higher (P<0.05) in TRIXcell+ extender as compared to Modena and GEPS at all hours of preservation which indicate that TRIXcell+ provided better protective action against acrosomal damage than Modena and GEPS extenders. It was also observed that the mean percentage of intact acrosome was significantly (P<0.05) higher in Modena than in GEPS at 72, 144 and 168 h of preservation. The present results were in close conformity with those reported by Kaeoket *et al.* (2010) but higher than that reported by Sa *et al.* (2013). The mean percentage of intact acrosome decreased significantly (P<0.05) with the increase in preservation time in all the extenders. This could be due to the 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 acrosome reaction (Johnson *et al.* 2000). There is increase in phospholipids and cholesterol in the seminal plasma on storage and high concentrations of these plasmatic components caused destructive changes in sperm

Table 1. Semen quality in different extenders during different hours of preservation at 15°C

Parameter	Extenders	Preservation period (h)						Overall
		0	72	96	120	144	168	
Sperm motility (%)	TRIXCell+	80.78^{Aa}±0.60	75.47^{Ba}±0.85	72.19^{Ba}±0.67	66.72^{Ca}±0.76	62.97^{Da}±0.70	61.72^{Da}±0.58	69.97±0.57
	Modena	79.06^{Aa}±0.79	69.84^{Bb}±1.02	62.66^{Cb}±1.25	56.25^{Db}±1.25	47.66^{Eb}±1.45	40.62^{Fb}±1.58	59.35±1.06
	GEPS	78.28^{Aa}±0.76	62.50^{Bc}±1.27	53.12^{Cc}±1.58	45.47^{Dc}±1.66	36.09^{Ec}±1.90	26.25^{Fc}±1.94	50.29±1.39
Intact acrosome (%)	TRIXCell+	89.01^{Aa}±0.58	85.27^{Ba}±0.73	81.69^{Ca}±0.95	77.65^{Da}±1.07	74.54^{Ea}±1.11	69.21^{Fa}±1.02	79.56±0.61
	Modena	88.63^{Aa}±1.05	82.02^{Bb}±1.02	76.22^{Cb}±1.12	71.89^{Db}±1.15	66.98^{Eb}±1.12	62.68^{Fb}±0.96	74.74±0.79
	GEPS	87.20^{Aa}±0.88	78.48^{Bc}±1.44	74.72^{Cb}±1.24	69.23^{Db}±1.27	63.03^{Ec}±1.18	58.50^{Fc}±1.08	71.86±0.84
HOST-reacted sperm (%)	TRIXCell+	66.90^{Aa}±1.10	61.91^{Ba}±0.93	56.28^{Ca}±0.94	50.21^{Da}±1.21	43.19^{Ea}±1.50	36.14^{Fa}±1.58	52.44±0.91
	Modena	64.16^{Aab}±0.94	56.21^{Bb}±0.93	49.32^{Cb}±1.23	43.08^{Db}±1.48	36.46^{Eb}±1.65	30.52^{Fb}±1.78	46.63±0.99
	GEPS	61.75^{Ab}±0.91	49.91^{Bc}±1.07	40.43^{Cc}±1.59	35.14^{Dc}±1.58	27.37^{Ec}±1.61	22.14^{Fc}±1.62	39.46±1.12

*32 observations. Means bearing different superscripts within row (large case) and column (smaller case) differ significantly (P<0.05).

membranes (Dimitrov *et al.* 2009). Further, gradual increase in the proportion of acrosomal damage with increase in hour of preservation could also be due to peroxidation effect (Jones and Mann 1977).

The mean percentage of HOST-reacted sperm was significantly higher (P<0.05) in TRIXcell+ as compared to Modena and GEPS extenders at all hours of preservation. The present observations were in close proximity with the reports of previous researchers (Saikia *et al.* 2016). However, Lalrintluanga *et al.* (2016) reported lower mean percentage of HOST-reacted sperm than obtained in the present study. The mean percentage of HOST-reacted spermatozoa decreased significantly (P<0.05) with the increase in duration of preservation in all the extenders which could be due to the progressive decrease in biochemical activity of spermatozoa with the increase in preservation period. This could also be explained by the fact that sperm have a high content of unsaturated fatty acids in their membranes (Johnson *et al*. 1969) and they lack a significant cytoplasmic component containing antioxidants (de Lamirande and Gagnon 1995, Saleh and Agarwal 2002). Therefore, sperms are highly susceptible to lipid peroxidation and excessive ROS formation by spermatozoa during preservation has been associated with damaging the plasma membrane (Kumaresan *et al.* 2009). In the present study, the percentage of spermatozoa responding to swelling was lower than the percentage of motility. The difference might be due to the fact that some spermatozoa with membrane damage remain motile (Zou and Yang 2000) or the membranes of some spermatozoa are inactivated when they come in contact with the hypo-osmotic solution.

Female pigs (40) were inseminated with 80 ml dose of semen held for 4 h at 22°C and preserved in TRIXcell+ extender at 15°C. The conception rate following AI with fresh diluted semen (N=25) and semen preserved for 24 (N=5), 48 (N=3), 72 (N=3), 96 (N=2) and 120 (N=2) h was 80, 80, 66.67, 66.67, 100 and 50%, respectively. van den Berg *et al.* (2014) also reported better farrowing rate and litter size for semen diluted in TRIXcell+ and stored for 5 days on farm fertility performance. However, in the present study, the data were inconclusive of the fertility efficacy of TRIXcell+ extender as the insemination number was very less and not done on equal number of females and hence, the result was variable for different age of semen used. Nevertheless, based on the present findings, it can be opined that insemination could be done successfully up to 5th day using semen extended with TRIXcell+ extender. Moreover, based on sperm motility results, semen preservation up to 168 h (7th day) could be possible as TRIXcell+ could maintain >60% sperm motility up to 168 h of preservation.

In conclusion, the quality of liquid semen based on sperm motility, intact acrosome and HOST-reacted sperm was significantly higher in TRIXcell+ than that in Modena and GEPS extenders for preservation of boar semen up to 168 h at 15ºC. We suggests definite proof of performance with respect to fertility on farm insemination trials using semen extended with TRIXcell+ up to 120 h of preservation.

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