The Pharma Innovation Journal 2017; 6(7): 1011-1014

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(7): 1011-1014

www.thepharmajournal.com
Received: 12-05-2017
Accepted: 13-06-2017

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Effect of extender and different storage temperature on keeping quality of crossbred LWY boar semen

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Abstract
Comparative efficacy of Farmer friendly Boar Semen Extender (FBSE), Normal Boar Semen Extender (NBSE) and PRIMXcell extender for preservation of boar semen up to 96 h was studied. Extended semen was stored at 5 ^{0}C in FBSE and at 15-18 ^{0}C in NBSE and PRIMXcell extenders. A total of 32 ejaculates (8 from each boar) from 4 crossbred LWY boars were collected. The overall mean per cent of individual motility, Live spermatozoa, Acrosome integrity, Hypo osmotic Swelling Test reacted spermatozoa and Morphologically normal spermatozoa of the semen after storage of 96 h in FBSE, NBSE and PRIMXcell was 67.65 ± 1.11, 60.00 ± 1.33 and 26.95 ± 2.03; 74.98 ± 0.77, 69.38 ± 1.00 and 58.35 ± 1.48; 90.10 ± 0.38, 91.20 ± 0.32 and 78.65 ± 0.82; 32.95 ± 1.06, 30.95 ± 1.13 and 12.38 ± 1.11 and 83.96 ± 0.38, 85.99 ± 0.40 and 87.40 ± 0.36, respectively. Overall mean pH of the extended semen was 6.67 ± 0.04, 6.87 ± 0.04 and 7.98 ± 0.03 in FBSE, NBSE and PRIMXcell extenders respectively. Individual motility and live spermatozoa were significantly ($p \leq 0.01$) higher in FBSE followed by NBSE, significantly low in PRIMXcell extender. The acrosome integrity and HOST reacted spermatozoa in FBSE and NBSE extenders were significantly ($p \leq 0.01$) differed from PRIMXcell extender. Significantly ($p \leq 0.01$) higher per cent of morphologically normal spermatozoa were observed in PRIMXcell extender followed by NBSE and FBSE. FBSE was found to be the superior extender for preservation followed by NBSE.

Keywords: Extenders, Crossbred LWY boar, Microscopic evaluation, Preservation

1. Introduction
Artificial insemination (AI) technique is yet to gain its popularity in pigs primarily because of lack of ambitious project and limited availability of superior boars to maintain a regular supply of preserved semen for insemination of sows maintained either in private or public sector. Most of the AI in the swine industry is being conducted on the day of semen collection or at the most 24 h post collection after fresh semen extended and stored at 15 to 18 °C. It will be more economical to the swine industry, if the preserved semen can be used for AI purposes up to 4 to 5 days after collection with optimal conception rate and litter size (Waterhouse *et al*., 2004) [25].

Preserving boar semen for short or prolonged period requires storage at 15-18 °C temperature (Correa *et al*., 2006) [7]. Keeping boar semen at 5 ºC was reported to be a cheaper alternative than liquid nitrogen to help with increasing the use of artificial insemination in the pig industry. Besides this, the bacterial growth reduces at 5 ºC, which would improve the quality of semen. The present investigation aimed to study the effect of extenders and storage temperature on keeping quality of boar semen.

Materials and Methods
Four crossbred Large White Yorkshire (LWY) boars aged 1-2 years stationed at Instructional Livestock Farm Complex (ILFC), College of Veterinary Science, Rajendranagar, Hyderabad were utilized for the study.

Semen collection was done by the gloved hand technique using adjustable dummy. The ejaculated semen was collected into a pre warmed (38 ^{0}C) glass beaker of 500 ml capacity covered with a double layered cheese cloth to separate gel fraction. A total of 32 ejaculates (8 ejaculates from each boar) were collected. In the first step, the semen sample was gently homogenized and divided into 3 equal parts and extended with preheated extenders at 37 ^{0}C for 1-2 hours namely Farmer friendly Boar Semen Extender (FBSE) (5 ^{0}C), (Developed by Central Coastal Agricultural Research Institute, ICAR, Goa, Patent serial number of FBSE is TEMP/E1/24427/MUM/2015), Normal Boar Semen Extender (NBSE) (Developed by Central

Coastal Agricultural Research Institute, ICAR, Goa), (patent serial number of NBSE is 3037/MUM/2015) and PRIMXcell (IMV technologies, France) (15-18 ^{0}C) in 1:1 ratio. The samples diluted with NBSE and PRIMXcell were stored in BOD incubator and the temperature was gradually decreased from 32 ^{0}C to 15-18 ^{0}C. The FBSE sample was kept at 4 ^{0}C. In the 2nd step, the semen samples of each extender was diluted to 3 billion sperms /dose (Each dose=60ml).

The extended semen was subjected to evaluation at 1/2, 24, 48, 72 and 96 h of preservation. The individual motility of spermatozoa. The per cent live spermatozoa (eosin-nigrosin stain - Bjorndahl *et al*., 2003) [5], Plasma membrane integrity (Hypo-osmotic swelling test- HOST- Jeyendran *et al.*, 1984) [11] and Acrosome integrity (Giemsa staining-Watson, 1975) [26]. pH (digital pH meter-Systronics, Ahmedabad) and abnormalities of spermatozoa (3% Rose Bengal stain-Pervage *et al.*, 2009) [19] were estimated as per standard procedures described by respective authors.

Results and Discussion

The results of different parameters are presented in Table 1 and Table 2. The overall per cent of individual motility of spermatozoa was significantly ($p \leq 0.01$) differed among extenders used. After 24 hours of storage, the individual motility was significantly ($p \leq 0.01$) differed among FBSE, NBSE and PRIMXcell and it was progressively decreased. In PRIMXcell, the per cent of sperm motility was drastically decreased as the duration of storage was increased and it was very low when compared to the other two extenders. (Table 1). Which are in line with the reports of Tyngkan *et al.* (2015) [23] in BTS, KIEV and LEY extenders. Variation in individual motility of extended semen might be due to storage temperature (Mapeka *et al.*, 2012) [18], breed (Stancic *et al*., 2011) [21], age of boar, rate of dilution (Lipensky *et al*., 2013) [17] and spermatozoa concentration in the ejaculate (Stancic *et al.*, 2011) [21]. The Reactive oxygen species showed to cause membrane deterioration, led to ATP depletion and decreased sperm movement (Armstrong *et al*., 1999) [3]. The per cent of motile spermatozoa decreased in all extenders as storage time increased and this could be correlated with variation in oxygen uptake and metabolic activity depended on the extender used.

Overall per cent of live spermatozoa was also significantly differed among extenders ($p \leq 0.01$). The per cent of live spermatozoa in FBSE and NBSE extenders at 1/2h, 24h, 72 h and 96 h of storage was significantly differed from PRIMXcell extender. But, a drastic decline ($p \leq 0.01$) in per cent live spermatozoa in PRIMXcell but after 24 h of storage and the difference recorded up to 96 h of storage. (Table 1). Variations in per cent of live spermatozoa in diluted semen was due to age of the boar, altered temperatures of storage (Khan *et al.*, 2015) [14, 15], progressive decline in nutrient content in extenders with increased period of preservation (Kommisrud *et al.*, 2002) [16] and alterations in the concentrations of compounds or ions (e.g. plasma proteins and K+) in diluted semen (Gadea, 2003) [10]. Adding an antibiotic at the appropriate concentration to an extender improves sperm survival and in turn fertility (Colenbrander *et al*., 1993) [6]. Cold shock can reduce motility as well as sperm vitality (Althouse *et al*., 1998) [2].

The overall per cent HOST reactive spermatozoa was significantly ($p \leq 0.01$) higher in FBSE and NBSE extenders when compared to PRIMXcell extender. Significantly ($p \leq 0.01$) less number of HOST reactive spermatozoa was recorded in PRIMXcell extender at each phase of study in storage (Table 1). The variation in HOST reacted spermatozoa per cent might be due to variation of biochemical constituents in seminal plasma (Barrios *et al*., 2000) [4] and transaminase activities in semen (Corteel, 1980) [8].

The overall per cent acrosomal integrity was significantly ($p \leq 0.01$) higher in FBSE and NBSE extenders when compared to PRIMXcell extender. The per cent of Acrosomal integrity in FBSE and NBSE extenders were significantly ($p \leq 0.01$) higher than PRIMXcell extender at each and every phase of preservation. (Table 1). Gradual increase in the proportion of acrosomal damage with increase in hour of preservation could be due to peroxidation effect (Pursel, 1979) [20]. There was increase in phospholipids and cholesterol in the seminal plasma on storage and high concentrations of these plasmatic components caused destructive changes in sperm membranes (Dimitrov *et al*., 2009) [9]. The decrease in acrosome integrity might thus be due to acrosome reaction in addition to membrane damage (Kommisrud *et al*., 2002) [16]. Acrosome integrity was affected by boar (Khan and Kumar, 2015) [14, 15] and pH >8.0 which lead to diminished sperm motility and an increased proportion of altered acrosomes (Althouse *et al*., 2000) [1]. Acrosomal integrity was also altered after holding, cooling, thawing etc. at various temperatures (Khan *et al*., 2015) [14, 15].

The pH of PRIMXcell extender was significantly ($p \leq 0.01$) towards alkaline side, while FBSE and NBSE was towards neutral side or slight acidic side. However, similar pattern of pH was also noticed among the overall mean of FBSE, NBSE and PRIMXcell extenders (Table 1). pH values were increased by 0.3 to 0.5 in first days of storage which decreases motility (Vyt *et al.*, 2004) [24]. pH values may vary according to the rate of dilution, depending on the influence of boar (Lipensky *et al.*, 2013) [17], storage time (Kaeok et *et al*., 2010) [12].

Overall mean revealed that significantly ($p \leq 0.01$) higher per cent of morphologically normal spermatozoa in PRIMXcell extender followed by NBSE and FBSE. In all extenders morphologically normal sperms were more or less maintained with little significant variation. The type of extender had no effect on occurrence of head abnormalities. There was significant difference ($p \leq 0.01$) in overall incidence of mid piece abnormalities among FBSE, NBSE and PRIMXcell extenders. In all three extenders, the incidence of mid piece abnormalities progressively increased as the duration of storage was increased. Overall mean per cent revealed that the higher per cent of tail abnormalities were noticed in FBSE followed by NBSE and PRIMXcell with significant difference ($p \leq 0.01$). There was no significant difference observed in total abnormalities during 24 to 96 h of storage (Table. 2). Karageorgiou *et al.* (2016) [13], reported lower per cent normal spermatozoa during storage of 1 to 2 days with various extenders than the present study.

It was concluded that FBSE was the superior extender to preserve boar semen followed by NBSE (15-18^{0}C), whereas, PRIMXcell extender could not preserve the boar semen even up to 48 h.

Acknowledgements

We are highly thankful to ICAR-CCARI, Goa, College of Veterinary Science, Rajendranagar, Hyderabad for providing necessary infrastructure facilities for this study and Sri venkateswara veterinary university for providing necessary financial support during the study.

Table 1: Effect of Extender and preservation time on various parameters in extended semen of crossbred LWY boar.

Parameter	Extender	1/2 h	24 h	48 h	72 h	96 h	Overall mean
Individual motility	F	79.21±1.29^{xa}	74.53±1.92^{xb}	68.43 ± 1.74^{xc}	60.78 ± 2.39^{xd}	55.31 ± 2.29^{xe}	67.65 ± 1.11^{x}
	N	77.96±1.41^{xa}	67.50±1.78^{yb}	59.21 ± 2.10^{yc}	50.46 ± 2.47^{yd}	44.84 ± 2.72^{ye}	60.00 ± 1.33^{y}
	P	66.40±2.15^{ya}	38.43±2.00^{zb}	20.46 ± 2.55^{zc}	7.50 ± 1.45^{zd}	1.96 ± 0.72^{ze}	26.95 ± 2.03^{z}
Live spermatozoa	F	83.00±0.83^{xa}	79.12±1.46^{xa}	75.62 ± 1.73^{xb}	70.81 ± 1.35^{xc}	66.34 ± 1.43^{xd}	74.98 ± 0.77^{x}
	N	79.46±1.81^{xa}	73.46±1.95^{xb}	67.71 ± 2.24^{yc}	66.46 ± 1.53^{xc}	59.81 ± 2.12^{xd}	69.38 ± 1.00^{y}
	P	75.03±1.84^{ya}	57.43±2.88^{yb}	57.71 ± 3.29^{zb}	52.03 ± 3.29^{yb}	49.53 ± 3.20^{yb}	58.35 ± 1.48^{z}
HOST reacted spermatozoa	F	52.28±1.66^{xa}	37.40±2.03^{xb}	27.62 ± 1.28^{xc}	25.87 ± 0.65^{xc}	21.37 ± 0.77^{xd}	32.95 ± 1.06^{x}
	N	50.40±2.04^{xa}	34.93±2.06^{xb}	27.78 ± 1.38^{xc}	22.21 ± 1.08^{yd}	19.43 ± 1.22^{xd}	30.95 ± 1.13^{x}
	P	36.31±1.13^{ya}	14.28±1.38^{yb}	6.93 ± 0.92^{yc}	2.81 ± 0.96^{zd}	1.59 ± 0.80^{yd}	12.38 ± 1.11^{y}
Acrosome integrity	F	94.75±0.26^{xa}	93.81±0.35^{xa}	90.00 ± 0.58^{xb}	86.90 ± 0.71^{xc}	8 5.06 ± 0.65^{xd}	90.10 ± 0.38^{x}
	N	95.34±0.33^{xa}	94.40±0.35^{xa}	91.0 ± 0.48^{xb}	88.96 ± 0.41^{xc}	86.31 ± 0.45^{xd}	91.20 ± 0.32^{x}
	P	90.34±0.52^{ya}	86.18±0.79^{yb}	77.09 ± 1.20^{yc}	72.50 ± 1.34^{yd}	67.15 ± 1.24^{ye}	78.65 ± 0.82^{y}
pH	F	6.57 ± 0.08^{xa}	6.60 ± 0.08^{xa}	6.71 ± 0.09^{xa}	6.75 ± 0.10^{xa}	6.74 ± 0.11^{xa}	6.67 ± 0.04^{x}
	N	6.81 ± 0.11^{xa}	6.80 ± 0.08^{xa}	6.87 ± 0.09^{xa}	6.92 ± 0.10^{xa}	6.95 ± 0.11^{xa}	6.87 ± 0.04^{y}
	P	7.74 ± 0.09^{ya}	7.83 ± 0.09^{ya}	7.96 ± 0.06^{yab}	8.13 ± 0.06^{ybc}	8.23 ± 0.06^{yc}	7.98 ± 0.03^{z}

Row wise bearing different superscripts (i.e. a, b, c…) differed significantly at p≤ 0.01
Column wise bearing different superscripts (i.e. x, y, z) differed significantly at p≤ 0.01
F- FBSE
N- NBSE
P- PRIMXcell

Table 2: Effect of Extender and preservation time on morphology of spermatozoa in extended semen of crossbred LWY boar.

Parameter	Extender	1/2 h	24 h	48 h	72 h	96 h	Overall mean
Normal spermatozoa	F	87.53±0.57^{xa}	84.90 ±0.83^{Xb}	83.65± 0.82^{xb}	82.71± 0.87^{xbc}	81.00± 0.75^{xc}	83.96±0.38^{x}
	N	89.65 ±0.61^{ya}	87.21±0.99^{XYb}	85.31± 0.99^{xbc}	84.00± 0.80^{xc}	83.78± 0.69^{yc}	85.99±0.40^{y}
	P	89.90 ±0.55^{ya}	87.75±0.66^{Yab}	88.21± 1.00^{yab}	86.43± 0.80^{ybc}	84.68± 0.76^{yc}	87.40 ±0.36^{z}
Head abnormalities	F	2.78 ± 0.18^{xa}	3.53 ± 0.24^{xab}	3.68 ± 0.35^{xab}	4.43 ± 0.39^{xb}	5.71 ± 0.52^{xc}	4.03 ± 0.17^{x}
	N	2.93 ± 0.17^{xa}	3.46 ± 0.28^{xab}	4.03 ± 0.37^{xbc}	4.68 ± 0.32^{xcd}	5.40 ± 0.38^{xd}	4.10 ± 0.17^{x}
	P	3.37 ± 0.33^{xa}	4.46 ± 0.47^{xab}	3.46 ± 0.30^{xabc}	4.40 ± 0.24^{xbc}	4.96 ± 0.35^{xc}	4.13 ± 0.16^{x}
Mid piece abnormalities	F	6.18 ± 0.43^{xa}	6.90 ± 0.63^{Xa}	9.09 ± 0.76^{Xb}	8.93 ± 0.65^{xb}	9.81± 0.67^{Xb}	8.18 ± 0.30^{x}
	N	5.78 ± 0.45^{xA}	6.40 ±0.63^{XAB}	7.53±0.71^{XYAB}	8.43±0.86^{xyB}	7.81±0.63^{YAB}	7.19 ± 0.30^{y}
	P	4.34 ± 0.35^{ya}	4.65 ± 0.41^{Yab}	6.09 ± 0.62^{Ybc}	6.71 ± 0.62^{ycd}	7.68 ± 0.50^{Yd}	5.90 ± 0.24^{z}
Tail abnormalities	F	3.50 ± 0.53^{xa}	4.59 ± 0.74^{xa}	3.68 ± 0.49^{xa}	3.87 ± 0.45^{Xa}	3.59 ± 0.44^{xa}	3.85 ± 0.24^{x}
	N	1.65 ± 0.21^{ya}	2.96 ± 0.49^{xab}	3.06 ± 0.51^{xyb}	2.68±0.46^{XYab}	3.03 ± 0.49^{xb}	2.68±0.204^{y}
	P	2.37 ± 0.32^{ya}	3.00 ± 0.46^{xa}	2.21 ± 0.35^{ya}	2.43 ± 0.37^{Ya}	2.71 ± 0.35^{xa}	2.55 ± 0.16^{y}

Row wise bearing different superscripts (i.e. a, b, c…) differed significantly at p≤ 0.01 and A, B, C…. differed significantly at p≤ 0.05.
Column wise bearing different superscripts (i.e. x, y, z) differed significantly at p≤ 0.01 and X, Y and Z differed significantly at p≤ 0.05.
F- FBSE
N- NBSE
P- PRIMXcell

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