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Effect of diluters, breeds and storage period on boar semen characteristics

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ABSTRACT

The study was conducted on 54 semen samples obtained from 9 boars comprising 3 each of Large White Yorkshire, Tamworth and T & D (cross of Tamworth and Desi) by split sample technique to study the effect of different dilutors, genetic groups and hours of preservation on spermatozoa characteristics. The dilutors used were Kiev, Modena and lactose egg yolk (LEY). The sperm motility % was significantly highest in lactose egg yolk dilutor at all the hours of preservations. Irrespective of dilutors, the sperm motility% was significantly highest in T & D boars. Live sperm count was significantly highest in lactose egg yolk dilutor than Kiev and Modena during the hours of preservation in all the boar breeds. The difference in head abnormality % was nonsignificant in all the breeds and dilutors at all the hours of preservation except at 96 h. Highly significant effect of both hours of preservation and dilutors on the incidence of tail abnormality was noted during the study. The % of tail abnormality revealed an increasing trend with increase in the duration of preservation. On dilutor wise comparison, it indicated lower incidence of tail abnormality with lactose egg yolk dilutor at all hours of preservation. Therefore, it may be concluded that LEY diluter can be successfully used for preservation of boar spermatozoa at 15°C in a BOD incubator up to 96 h.

Key words: Boar, Diluter, Kiev, Lactose egg yolk, Modena, Pig, Semen preservation

Recent studies indicated that the boar has a significant influence on conception rate, litter size, birth weight and survival of piglets, the effect being due both to genetic factors and variation in semen quality. Therefore, evaluation of semen quality at ejaculation as well as at utilization time is essential for improving the fertility and prolificacy of the sow. The fertility of semen bears a high degree of relationship with the various abnormalities of spermatozoa. Different extendors of boar semen also affect the seminal attributes (Speight *et al.* 2012 and Khan *et al.* 2012*).* In India, very limited work on evaluation and preservation of boar semen (Rao *et al.* 1992) has been conducted. Thus, most of the aspects related to evaluation and preservation of boar semen has remained unexplored. Therefore, the present study has been planned to study selection of suitable dilutor for better preservation of semen in different genetic groups of pig.

MATERIALS AND METHODS

Semen samples (54) obtained from 9 boars comprising 3 each of Large White Yorkshire, Tamworth and T & D (Tamworth×Desi) were used by split sample technique for studying the effect of different dilutors as well as preservation time on preservability of spermatozoal characteristics and enzyme activities of semen during preservation at 15°C. The dilutors used were Kiev (Johnson *et al.* 1981), Modena (Sone *et al.* 1992) and Lactose egg yolk (Park and Purcell 1985). The original dilutors were slightly modified by replacing the antibiotics used in the dilutors with gentamicin sulphate alone in the present study. The semen samples were collected twice in a week from each boar by gloved hand technique. The sperm rich portion of the ejaculate was collected in preheated sterilized thermos flask of 500 ml capacity. The opening of the flask was covered with clean and sterilized muslin cloth. At the end of collection the muslin cloth with gel mass was removed and the thermos was capped immediately. Immediately after collection the semen samples were brought to the laboratory and evaluated within 1h of collection. Initial motility and sperm abnormalities were assessed following the procedure of Hancock (1959). The % of live spermatozoa was determined with the help of eosin-nigrosin stain. Concentration of spermatozoa was estimated by haemocytometer method. All the constituents of dilutors except egg yolk in lactose egg yolk dilutor were mixed and kept overnight at 5°C in a refrigerator. Just before collection of semen the extenders were warmed to 37°C and the egg yolk (20%) was added in case of lactose egg yolk dilutor.

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Immediately after collection, the thermos flask containing the semen was tightly closed and brought to the laboratory. At about 15–20 min after collection each ejaculate was split into 3 parts and was diluted @ 1:8 with the 3 dilutors. The diluted semen was then filled in (20 ml) capacity glass vials having rubber stopper and was preserved at 15°C in a BOD incubator up to 96 h. For each dilutor per ejaculate one vial with extended semen was preserved. The preserved semen samples were evaluated at 24, 48, 72 and 96 h of preservation. The results were analyzed statistically according to Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Motility of spermatozoa

During the present study, the sperm motility % was significantly highest in lactose egg yolk dilutor at all the hours of preservations (Table 1). Further critical difference test revealed that the sperm motility % irrespective of breed differed significantly between the dilutors at all the hours of preservation. However, no significant difference was observed between Kiev and Modena dilutors at 96 h of preservation. This finding is in agreement with the results of Rao *et al.* (1992), Lalrintluanga *et al.* (2002) and Khan *et al.* (2012). It further revealed that the motility % was significantly higher in lactose egg yolk dilutor than Kiev and Modena at all the hours of preservation. Lalrintluanga *et al.* (2002), who also reported higher motility percent in Lactose egg yolk dilutor than the other dilutors used in their studies. The higher sperm motility % in lactose egg yolk dilutor might be due to the presence of egg yolk and higher concentration of sugar in this dilutor. Lalrintluanga *et al.* (2002) and Khan *et al.* (2012) found egg yolk beneficial for preservation of boar semen. In lactose egg yolk dilutor the concentration of lactose was 7.0 % whereas in other 2 dilutors the sugar was glucose and its concentration varied from 2.75 to 6.0 %. The higher sperm motility in lactose egg yolk dilutor without buffering agent recorded in this study confirms the finding of Arhipovec (1966), who observed higher sperm motility in glucose egg yolk dilutor without buffering agent.

Irrespective of dilutors, the sperm motility % was significantly higher in T & D boars followed by Large White Yorkshire and Tamworth at all the hours of preservation except at 48 and 96 h of preservation where the motility % of spermatozoa of Large White Yorkshire and Tamworth boars did not differ significantly from each other (Table 2). The available literatures suggested that very little work has been conducted on this line in boars so valid comparison could not be made with the present findings.

Live sperm count

Live sperm count was significantly ($P<0.01$) higher in lactose egg yolk dilutor than Kiev and Modena at all the hours of preservation in all the breeds of boar (Table 1).

Irrespective of breeds also, the live sperm count was significantly more in lactose egg yolk dilutors than Kiev and Modena at all the hours of preservation. This is in agreement with the findings of Khan *et al.* (2011) and Khan *et al.* (2012). The higher live sperm count in lactose egg yolk dilutor might be due to the presence of egg yolk and higher concentration of sugar (lactose) in this dilutor. In Kiev and Modena dilutor, egg yolk was not present and the concentration of sugar (glucose) was also less than that in lactose egg yolk dilutor (Lalrintluana *et al.* 2002). All the 3 dilutors differed significantly among themselves up to 72 h whereas at 96 h, it did not vary significantly between Kiev and Modena dilutors. This difference might be due to the difference in composition of the dilutors. Analysis of variance conducted over pooled data (Table 2) revealed that irrespective of

Table 1. Dilutor wise average seminal attributes at different hours of preservation

Period of preservation (h)	Dilutors	Sperm motility %	Live sperm %	Sperm head abnormality %	Sperm tail abnormality %
0	KIEV	80.29±0.26^{b}	84.20 ±1.148	2.81±0.200	2.49±0.214^{b}
	MODENA	79.59±0.253^{a}	83.33±0.242	2.94±0.197	2.58±0.228^{b}
	LEY	81.81±0.244^{c}	85.07±0.231	2.807±0.187	1.98±0.215^{a}
24	KIEV	67.96±0.197^{b}	73.42±0.245^{b}	2.83±0.248	3.21±0.202^{a}
	MODENA	65.02±0.197^{a}	71.61±0.238^{a}	2.74±0.230	4.57±0.048^{b}
	LEY	71.24±0.196^{c}	76.22±0.272^{c}	2.61±0.203	3.20±0.21^{a}
48	KIEV	58.76±0.250^{b}	64.85±0.253^{b}	2.54±0.226	5.92±0.192^{b}
	MODENA	56.94±0.232^{a}	63.87±0.256^{a}	2.91±0.204	6.89±0.184^{c}
	LEY	64.19±0.236^{c}	69.81±0.237^{c}	2.58±0.216	5.19±0.176^{a}
72	KIEV	52.27±0.214^{b}	58.19±0.226^{a}	2.76±0.234	11.13±1.689^{ab}
	MODENA	51.38±0.200^{a}	57.52±0.303^{a}	2.86±0.204	11.72 ±0.321^{b}
	LEY	57.78±0.297^{c}	62.20±0.251^{b}	2.70±0.213	10.43±0.203^{a}
96	KIEV	46.54±0.251^{a}	52.33±0.188^{a}	2.72±0.386^{ab}	16.45±0.156^{a}
	MODENA	45.98±0.192^{a}	52.15±0.247^{a}	2.84±0.188^{b}	18.27±0.43^{b}
	LEY	49.69±0.205^{b}	56.89±0.275^{b}	2.65±0.204^{a}	16.28±0.166^{a}

Each value is the average of 54 observations. Mean under the same superscript with in a column did not differ significantly.

Table 2. Breed wise average seminal attributes at different hours of preservation

Period of preservation (h)	Breed	Sperm motility %	Live sperm %	Sperm head abnormality %	Sperm tail abnormality %
0	LARGE WHITE YORKSHIRE	81.42±0.20[b]	83.41 ± 0.167	2.79 ± 0.182	2.24 ± 0.226
	TAM WORTH	78.04±0.197[a]	83.01 ± 0.220	2.88 ± 0.192	2.37 ± 0.229
	T & D	82.24 ± 0.197[c]	86.19 ± 1.152	2.88 ± 0.212	2.45 ± 0.232
24	LARGE WHITE YORKSHIRE	67.50 ± 0.25[b]	72.69 ±0.224[a]	2.62 ± 0.202	3.54±0.232
	TAM WORTH	66.63±0.257[a]	72.11±0.239[a]	2.78±0.226	3.72±0.251
	T & D	70.09±0.277[c]	76.46±0.239[b]	2.77±0.253	3.67±0.259
48	LARGE WHITE YORKSHIRE	58.52±0.28[a]	64.79±0.247[a]	2.64±0.196	5.98±0.203
	TAM WORTH	58.28±0.303[a]	64.37±0.286[a]	2.70±0.227	6.05±0.227
	T & D	63.09±0.300[b]	69.37±0.882[b]	2.69±0.235	5.97±0.221
72	LARGE WHITE YORKSHIRE	53.61±0.318[b]	58.43±0.312[a]	2.68±0.189	11.26±0.233
	TAM WORTH	51.70±0.253[a]	58.06±0.283[a]	2.89±0.215	11.22±0.267
	T & D	56.13 ± 0.326[c]	61.42±0.307[b]	2.75±0.245	10.81±0.293
96	LARGE WHITE YORKSHIRE	46.85±0.241[a]	54.31±0.346[b]	2.56±0.207	16.94±0.198
	TAM WORTH	46.04±0.265[a]	51.39±0.261[a]	2.76±0.255	17.08±0.214
	T & D	49.31±0.193[b]	55.67±0.179[c]	2.89±0.369	16.98±0.228

Each value is the average of 54 observations. Mean under the same superscript within a column did not differ significantly.

dilutors, the live sperm % was significantly highest in T&D boars. The live sperm count was significantly higher in Large White Yorkshire than Tamworth at 24 and 72 h, but did not vary between themselves at 48 and 96 h of preservation. Significant difference (P<0.01) observed between boars for live sperm % was reported by Tamuli *et al.* (1986). Significant difference observed between boars for this attributes might be due to individual difference in their inherent qualities.

Head and tail abnormalities

During the present study the difference in head abnormality % was nonsignificant in all the breeds and dilutors at all the hours of preservation except at 96 h. Irrespective of breed, the sperm head abnormality % did not vary significantly at all the hours of preservation except at 96 h where the head abnormality % was lowest in lactose egg yolk dilutors. Irrespective of dilutors, the head abnormality % did not vary significantly between the breeds at any hr of preservation. The present finding is in consonance with Pandey (1993) who also recorded nonsignificant effect of dilutors on the occurrence of sperm head abnormality in boars.

Tail abnormalities (%) varied from 1.98 ± 0.215 to 18.27 ± 0.430 in different dilutors (irrespective of breed) and 2.24 ± 0.226 to 17.08 ± 0.214 in different breed (irrespective of dilutors) at 0, 24, 48, 72 and 96 h of preservation. Highly significant effect of both hours of preservation and dilutors on the incidence of tail abnormality was noted during the study. The % of tail abnormality revealed an increasing trend with increase in the duration of preservation. Dilutor-wise comparison indicated lower incidence of tail abnormality with lactose egg yolk dilutor at all hours of preservation. Increase in the incidence of tail abnormalities after preservation up to 72 h had also been reported by Pandey (1993), which supported the present findings.

In summary, sperm motility and live sperm % varied significantly among dilutors and breeds at all the hours of preservation. Irrespective of breed, sperm motility and live sperm % was found to be significantly (P<0.01) higher in lactose egg yolk followed by Kiev and Modena dilutors at all the hours of preservation. Irrespective of dilutors sperm motility and live sperm % was significantly (P<0.01) higher in T & D than the Large White Yorkshire and Tamworth boars. percent of live sperm varied significantly among dilutors and breeds at all the hours of preservation. Nonsignificant difference could be observed in % of sperm head and tail abnormalities among the breeds at any hour of preservation. Irrespective of breed sperm head abnormality % was significantly lowest in lactose egg yolk dilutor at 96 h of preservation. Irrespective of breed sperm tail abnormality % was significantly (P< 0.01) higher in Modena as compared to Kiev and Lactose egg yolk dilutors at 24, 48 and 96 h of preservation.

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