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EFFECT OF DILUTORS, BREED AND PRESERVATION TIME ON EXTRACELLULAR ENZYMATIC ACTIVITY OF PRESERVED BOAR SEMEN

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KEYWORDS

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Preservation

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ABSTRACT

Semen samples were collected from breeds of boars comprising Large White Yorkshire, Tamworth and Cross of Tamworth and Local breed (T & D) breeds. Experimentation was conducted by collecting 216 semen samples from nine boars. After evaluation of neat semen, samples were diluted with Kiev, Modena and Lactose Egg Yolk diluters (LEY) subsequently preserved at 15°C in BOD incubator for up to 96 hours. Diluted semen samples were evaluated at different hours of interval starting at 24 hours interval up to 96 hours for extra-cellular enzyme activity of Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT). In neat semen significant ($P<0.01$) effect of breed on extracellular activity of AST and ALT was recorded and it was lower in T&D boar. Mean extracellular level of AST and ALT did not vary significantly in any of these diluters and breeds during observed hours of preservation. Irrespective of breeds, extracellular levels of AST and ALT were significantly lower in LEY followed by Kiev and Modena diluters at 24, 48, 72 and 96 hours of preservation. These diluters varied significantly at 72 and 96 hours duration of preservation among themselves. Whereas at 24 and 48 hours of preservation, they did not vary significantly between Kiev and LEY diluters. It was found that irrespective of diluters, AST and ALT level differed significantly between breeds only at 0 hours of preservation. At other hours of preservation difference in levels of AST and ALT was not found significant between breeds.

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1 Introduction

Pig is the highest litter bearing capacity among meat producing livestock species. They have faster growth rate, shorter generation interval, and higher dressing percentage along with high feed conversion efficiency. Hence, pig enterprise is one of the important solutions to bridge the gap between demand and supply of animal protein. Scarcity of superior boars and high cost of rearing them for small and marginal farmers necessitate use of Artificial Insemination (A.I.). This technique is a scientific breeding tool for livestock production and for rapid multiplication of germplasm of superior boars. The practice of artificial insemination on commercial basis exists in some of countries but in India, its application in pig breeding is very limited.

Although sow has considerable influence on conception rate, litter size; recent studies indicated boar has significant influence over it as well as on birth weight, survival of piglets due to genetic factors and variation in semen quality (Skjervold, 1963; Rasbech, 1969). Therefore, evaluation of semen quality at ejaculation as well as at utilization time is essential for improvement in fertility and prolificacy of sow. Microscopic evaluation of spermatozoa does not provide all information necessary to explain potential fertility of male. Study of certain enzymes had shown functional significance in evaluation of male fertility. Highly significant negative correlation between Lactic Dehydrogenase (LDH) activity and non-return conception rate has been observed. Positive correlation between AST activity and fertilizing ability of spermatozoa has been recorded (Pangawkar et al., 1988). Leakage of enzyme from spermatozoa subjected to preservation is accompanied by lowering of their biological value and this phenomenon corresponds to morphological state of sperm structures.

Several extenders are currently in use throughout the world by commercial artificial insemination centers. Among them, Kiev is the most widely used dilutor. In some experiments Belt's Ville Thaw solution (BTS) has given better fertility results than Kiev. In spite of sufficient literature available on different characters of semen yet no single characteristics has been identified which could serve as trustworthy indicator of its fertility. Therefore, semen sample evaluated on the basis of different parameters must be substantiated by fertility rate.

2 Materials and Methods

2.1 Experimental Animals

Present study was conducted on semen samples obtained from three Large White Yorkshire, three Tamworth and three T&D boars (of approximately 2-3 years of age) belonging to Government Pig Breeding Farm, Kanke and Pig Breeding Farm, Ranchi. Veterinary College, Kanke, Ranchi. Boars were maintained under identical ration schedule and management

conditions. They were trained for semen collection on oestrus sows.

2.2 Collection of Semen

Semen samples were collected twice a week from each boar by gloved hand technique following the procedure of Zavos & Liptrap (1987). After observing positive response of mounting on restrained estrus female, boar was used for semen collection. The sperm rich portion of the ejaculate was collected in preheated sterilized thermos flask of 500 ml capacity at 40°C. The opening of flask was covered with clean and sterilized muslin cloth. At the end of collection muslin cloth with gel mass was removed and thermos was capped immediately.

2.3 Processing of Semen

A total of 54 semen samples (6 ejaculates obtained each from 3 Large White Yorkshire, 3 Tamworth and 3 T & D boars) were used by split sample technique for studying effect of different dilutors, preservation time on preservability of spermatozoal characteristics and enzyme activities of semen during preservation at 15° C. Dilutors used were Kiev (Johnson et al., 1981), Modena (Sone et al., 1992) and Lactose egg yolk (Park & Pursel, 1985). Original dilutors were slightly modified by replacing antibiotics used in dilutors with Gentamicin sulphate alone in the present study.

Various constituents of dilutors were mixed and kept overnight at 5°C in a refrigerator except egg yolk in Lactose egg yolk dilutor. Semen extenders were warmed to 37°C and egg yolk was added in case of Lactose egg yolk dilutor before collection of semen. Immediately after collection, thermos flask containing semen was tightly closed and brought to laboratory. After 15-20 minutes from collection time, each ejaculate was split into 3 parts and was diluted at the rate of 1:8 with these three dilutors. The diluted semen was later filled in (20 ml) capacity glass vials having rubber stopper and preserved at 15°C in a BOD incubator up to 96 hours. For each dilutor per ejaculate one vial with extended semen was preserved. The preserved semen samples were evaluated at 24, 48, 72 and 96 hours of preservation for enzymatic estimation.

2.4 Enzyme estimation

Activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were estimated in seminal plasma of undiluted, freshly diluted and preserved semen samples. The preserved semen samples were taken out from B.O.D. incubator (15°C), warmed up to 37°C and then centrifuged at 1500 rpm for 20 minutes to obtain seminal plasma. Eighteen samples from each boar were utilized for assay of these enzymes. The enzyme activity was calculated according to procedure followed by Span Diagnostic Private Limited, Surat (India).

2.5 Aspartate aminotransferase (AST)

Activity of AST was estimated using diagnostic reagent kit supplied by Span Diagnostics Private Limited, Surat, by the method of Reitman & Frankel (1957). These were Reagent 1 - Buffered Aspartate - α - KG substrate, pH 7.4; Reagent 2 - DNPH Colour Reagent; Reagent 3 - Sodium hydroxide, 4 N; Reagent 4 -Working Pyruvate Standard 2 mM;

Solution I was prepared by diluting 1 ml of Reagent 3 to 10 ml distilled water. Reagent 1,2 and 4 did not require any preparation.

3 Results

The mean values of extracellular activity of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in different breeds and dilutors at different hours of preservation were presented in Tables 1, 2, 3 and 4. The results of analysis of variance were presented in Tables 1a and 2a.

3. 1 Effect on Aspartate Amino Transferase

Breed wise activities of AST in preserved semen samples in different dilutors were depicted under Table 1. Non-significant effect of interactions between breeds and dilutors at studied hours of preservation was noticed. Analysis of variance revealed significant ($P < 0.01$) variation between breed at 0 hour and dilutors had significant effect at different hours of preservation except at 0 hour. The influence of dilutors irrespective of breed was significant ($P < 0.01$). The level of AST enzyme was significantly higher (Table 3) in Modena dilutor than Kiev & LEY dilutors at all the hours of preservation. Critical difference test (Table-3) revealed that AST level irrespective of breed was significantly higher in

Modena than in Kiev and LEY dilutor at all the hours of preservation. All the three dilutors differed significantly among themselves (Table 3) at 72 and 96 hours of preservation. Whereas at 24 and 48 hours of preservation they did not vary significantly between Kiev and LEY dilutors. It further revealed that irrespective of dilutors (Table 4) AST level differed significantly between breeds only at 0 hours of preservation which was significantly highest 182.58 ± 1.49 units/ 10^9 sperms in Large White Yorkshire followed by 176.57 ± 1.90 units/ 10^9 sperms in Tamworth & 174.79 ± 1.87 units/ 10^9 sperms in T & D boars.

3. 2 Effect on Alanine Amino Transferase

Breed wise enzymatic level of ALT in different dilutors & breeds has been furnished under Table-2. Significant ($p < 0.01$) effect of dilutors on the level of ALT enzyme in all three breeds at different hours of preservation. The effect of breed on ALT level was also significant ($P < 0.01$) while the effect of interaction between breed and dilutors were found non-significant at varied hours of preservation. The influence of dilutors irrespective of breed was significant ($P < 0.01$). Critical difference test (Table-3) revealed that ALT level irrespective of breed was significantly higher in Modena than Kiev, Lactose egg yolk dilutors at different hours of preservation. But it did not show any significant difference between Kiev and Lactose egg yolk dilutors. The study further revealed that irrespective of dilutors, ALT level was significantly highest in Large White Yorkshire 45.39 ± 0.37 units/ 10^9 sperms followed by Tamworth (41.91 ± 0.69 units/ 10^9 sperms) and T & D boars (39.81 ± 0.67 units/ 10^9 sperms) at 0 hours of preservation only (Table-4). At all other hours of preservation difference between levels of ALT was not significant between breeds.

Table 1 Mean extracellular AST activity (units/ 10^9 Sperms) of boar semen during preservation with different dilutors*.

PERIOD OF PRESERVATION	BREED	DILUTORS		
		KIEV	MODENA	LEY
0 Hour	LARGE WHITE YORKSHIRE	184.61 $\pm$ 1.40	182.67 $\pm$ 3.12	180.45 $\pm$ 2.92
	TAM WORTH	177.22 $\pm$ 2.75	177.67 $\pm$ 3.39	174.83 $\pm$ 3.82
	T & D	175.28 $\pm$ 2.69	175.94 $\pm$ 3.35	173.17 $\pm$ 3.75
24 Hour	LARGE WHITE YORKSHIRE	343.06 $\pm$ 7.74	511.44 $\pm$ 2.53	346.5 $\pm$ 6.08
	TAM WORTH	347.89 $\pm$ 7.21	508.78 $\pm$ 2.74	344.5 $\pm$ 6.45
	T & D	342.17 $\pm$ 7.5	506.94 $\pm$ 2.07	339.83 $\pm$ 6.09
48 Hour	LARGE WHITE YORKSHIRE	627.07 $\pm$ 9.14	683.11 $\pm$ 3.55	626.89 $\pm$ 7.09
	TAM WORTH	632.33 $\pm$ 8.31	682.11 $\pm$ 3.10	628.67 $\pm$ 6.11
	T & D	630.61 $\pm$ 8.26	679.17 $\pm$ 3.22	626.72 $\pm$ 6.14
72 Hour	LARGE WHITE YORKSHIRE	752.45 $\pm$ 7.39	824.5 $\pm$ 4.86	749.61 $\pm$ 6.54
	TAM WORTH	762.72 $\pm$ 4.63	822.00 $\pm$ 4.77	746.67 $\pm$ 6.77
	T & D	758.67 $\pm$ 5.08	820.28 $\pm$ 4.72	744.22 $\pm$ 6.87
96 Hour	LARGE WHITE YORKSHIRE	789.56 $\pm$ 7.41	864.11 $\pm$ 5.45	785.00 $\pm$ 7.59
	TAM WORTH	800.56 $\pm$ 5.55	864.67 $\pm$ 4.94	785.17 $\pm$ 7.03
	T & D	798.5 $\pm$ 5.69	863.00 $\pm$ 4.89	782.61 $\pm$ 7.08

*Each value is average of 18 observations

Table 2 Mean extracellular ALT activity (units/10⁹ Sperms) of boar semen during preservation with different dilutors*.

PERIOD OF PRESERVATION	BREED	DILUTORS		
		KIEV	MODENA	LEY
0 Hour	LARGE WHITE ORKSHIRE	43.78 ± 0.50	44.61 ± 0.52	47.78 ± 0.50
	TAM WORTH	40.11 ± 1.24	41.33 ± 1.08	44.28 ± 1.16
	T & D	37.89 ± 1.22	39.17 ± 1.01	42.39 ± 1.00
24 Hour	LARGE WHITE ORKSHIRE	94.33 ± 2.33	103.44 ± 2.84	93.83 ± 2.18
	TAM WORTH	92.28 ± 2.47	101.89 ± 2.71	91.61 ± 2.43
	T & D	90.78 ± 2.43	99.67 ± 2.86	89.67 ± 2.46
48 Hour	LARGE WHITE ORKSHIRE	144.94 ± 3.74	159.11 ± 4.52	149.44 ± 2.89
	TAM WORTH	143.78 ± 3.64	157.78 ± 4.49	147.44 ± 3.05
	T & D	142.28 ± 3.66	155.78 ± 4.43	145.94 ± 2.79
72 Hour	LARGE WHITE ORKSHIRE	193.00 ± 3.08	206.61 ± 4.94	188.33 ± 5.19
	TAM WORTH	191.17 ± 3.06	221.61 ± 7.54	186.56 ± 5.11
	T & D	189.5 ± 3.00	202.83 ± 4.84	184.5 ± 5.05
96 Hour	LARGE WHITE ORKSHIRE	208.61 ± 2.36	222.44 ± 5.09	203.67 ± 5.37
	TAM WORTH	206.28 ± 2.32	239.72 ± 7.46	202.11 ± 5.39
	T & D	204.83 ± 2.30	220.94 ± 4.80	200.22 ± 5.30

*Each value is the average of 18 observations.

4 Discussions

4.1 Aspartate Aminotransferase (AST)

This enzyme is located primarily in mid piece of sperm cell (Mann & Mann, 1981) and measurement of its release is considered to be a sensitive indicator of sperm damage occurring during preservation (Pursel et al., 1970; Brown et al., 1971; Bower et al., 1973; Forejteck & Navratil 1984). Ciereszko et al. (1992) also reported that GOT/AST release into extracellular medium was best indicator of cell damage. High correlation coefficient between AST activity and fertilizing ability of spermatozoa (Pangawkar et al., 1988) has been recorded. Leakages of enzymes from spermatozoa subjected to preservation were accompanied by lowering of their biological

value. Boar semen contains 0.86 ± 0.04 mg percent of ammonia (Khomyak, 1984) and functional significance of transaminases was attributed to their ability to decrease level of ammonia produced by de-amination of adenylyl derivatives. On perusal of Table-3, significantly lowest value of enzyme was recorded in Lactose Egg Yolk followed by Kiev and Modena dilutors. Irrespective of dilutors, extracellular AST level was significantly not different in any breeds of boar. Pandey (1993) reported significant effect of dilutors on extracellular activity of AST in boar semen. Higher and lower value of extracellular enzyme with different dilutors reflected protective properties of ingredients added in dilutor. Increasing trend in AST level with increasing hours of preservation with three dilutors was observed in the present study, which is similar to findings of Azawi et al. (1990) and Pandey & Singh (2001).

Table 3 Dilutor wise average enzymatic activity at different hours of preservation (Irrespective of breeds)*.

Period of preservation	Dilutors	AST Units/10 ⁹ sperms	ALT Units/10 ⁹ sperms
0 Hour	KIEV	179.04 ± 1.45 ^a	41.70 ± 0.59 ^a
	MODENA	178.76 ± 1.90 ^a	44.82 ± 0.62 ^b
	LEY	176.15 ± 2.04 ^a	40.59 ± 0.68 ^a
24 Hour	KIEV	344.37 ± 4.25 ^a	92.46 ± 1.38 ^a
	MODENA	509.05 ± 1.41 ^b	101.67 ± 1.60 ^b
	LEY	343.61 ± 3.54 ^a	91.70 ± 1.36 ^a
48 Hour	KIEV	630.00 ± 4.86 ^a	147.61 ± 1.66 ^a
	MODENA	681.46 ± 1.87 ^b	157.56 ± 2.54 ^b
	LEY	627.43 ± 3.66 ^a	143.67 ± 2.09 ^a
72 Hour	KIEV	757.95 ± 3.35 ^b	191.22 ± 1.73 ^a
	MODENA	822.26 ± 2.72 ^c	210.35 ± 3.52 ^b
	LEY	746.83 ± 3.82 ^a	186.46 ± 2.91 ^a
96 Hour	KIEV	796.21 ± 3.61 ^b	206.57 ± 1.33 ^a
	MODENA	863.90 ± 2.89 ^c	227.70 ± 3.54 ^b
	LEY	784.26 ± 4.10 ^a	202.00 ± 3.04 ^a

*Mean value is the average of 54 observations; Mean under same super script in a column did not differ significantly.

Table 4 Breed wise average enzymatic activity at different hours of preservation (Irrespective of dilutors).

Period of preservation	Breed	AST Units/10 ⁹ sperms	ALT Units/10 ⁹ sperms
0 Hour	LARGE WHITE YORKSHIRE	182.58±1.49 ^b	45.39±0.37 ^c
	TAMWORTH	176.57±1.90 ^a	41.91±0.69 ^b
	T & D	174.79±1.87 ^a	39.81 ± 0.67 ^a
24 Hour	LARGE WHITE YORKSHIRE	400.33±11.29 ^a	97.20±1.52 ^a
	TAMWORTH	400.39±11.05 ^a	95.26±1.58 ^a
	T & D	396.30±11.22 ^a	93.37 ± 1.59 ^a
48 Hour	LARGE WHITE YORKSHIRE	645.69±5.36 ^a	151.16±2.29 ^a
	TAMWORTH	647.70±4.86 ^a	149.67 ± 2.29 ^a
	T & D	645.5 ± 4.81 ^a	148.00 ± 2.23 ^a
72 Hour	LARGE WHITE YORKSHIRE	775.52±5.97 ^a	195.98 ± 2.76 ^a
	TAMWORTH	777.13±5.42 ^a	199.78 ± 3.79 ^a
	T & D	774.39±5.54 ^a	192.28 ± 2.71 ^a
96 Hour	LARGE WHITE YORKSHIRE	812.89±6.33 ^a	211.57 ± 2.76 ^a
	TAMWORTH	816.80±5.79 ^a	216.04 ± 3.87 ^a
	T & D	814.7 ± 5.85 ^a	208.66 ± 2.74 ^a

* Mean value is the average of 54 observations; Mean under same super script in a column did not differ significantly.

4.2 Alanine aminotransferase (ALT)

ALT, an enzyme of transaminase group has significant correlation with percentage of motile spermatozoa, sperm concentration and fertility (Khokhar et al., 1987). Significant effect of dilutors on ALT level was reported by Pandey & Singh (2001). The assay of enzyme has been widely used to detect sperm cell damage and found to be more sensitive indicator for assessing membrane damage in boar sperm than ultrastructural investigation. Mean value of ALT in preserved semen with three dilutors and three breeds at 24, 48, 72 and 96 hours of preservation were presented in Table 3 and 4. Perusal of Table - 3 revealed that dilutors used during this study had significant effect on ALT activity at all hours of preservation. This was lowest in Lactose Egg Yolk followed by Kiev and Modena dilutors. Irrespective of dilutors, non-significant effect of breed was observed at all hours of preservation on extracellular activity of ALT.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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