

Enzymatic Activities in Fresh Seminal Plasma and Extended Refrigerated Semen in Nari Suvarna Rams

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Abstract

Enzyme activities in the seminal plasma are the good indicators of fertility. A study was conducted to determine the activities of certain enzymes in fresh seminal plasma and extended refrigerated semen in Nari Suvarna rams. Six rams of Nari Suvarna breed were selected and sixteen ejaculates were collected from each ram for a period of eight weeks at the rate of two collections per week by artificial vagina technique. The seminal plasma was separated by centrifugation. Enzyme activities such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were estimated. There was a non-significant ($P>0.05$) difference between the activities of enzymes in seminal plasma except ALP activity between the rams. The semen was extended using Tris and citrate buffers and the enzymatic activities such as ALP, ALT, AST and LDH were determined after refrigeration for 24 hrs for four Nari Suvarna rams. The numerically increased activities of all the enzymes were observed after extension and refrigeration. It was concluded that the levels of ALP, ALT, AST and LDH enzymes were established in the seminal plasma of Nari Suvarna rams in the present study and the numerical increase in the activities of these enzymes after refrigeration with extension of Tris and citrate buffers for 24 hours could be due to damage to the sperm membrane and leakage of enzymes in to the seminal fluid.

Keywords: Nari Suvarna rams, seminal plasma, enzymatic activities, Tris buffer, Citrate buffer

The Nari Suvarna or Nari Suvarna breed of sheep has been developed at Nimbkar Agricultural Research Institute (NARI), Maharashtra in 1990 by introducing the *FecB* (*Booroola*) gene from the Garole breed of Sunderbans

regions, West Bengal. FecB gene increased ovulation rate and litter size in multiparous ewes (Nimbkar *et al.* 2003). Nari Suvarna sheep have either 9:1 breed proportion of Deccani and Garole or 6:3:1 breed proportion of Deccani, Madgyal and Garole and the ewes in both the cases are capable of producing and raising twin lambs. Nari Suvarna being a new breed, the studies on semen and seminal plasma is the need of present time.

Studies on biochemical components of seminal plasma are one of the rapidly expanding fields of research, particularly concerning the biological significance of the various constituents. When sperm cells are exposed to adverse conditions, seminal plasma can become a hostile medium for them. Better understanding of biochemical properties of the components of seminal plasma could help in formulation of extenders and improvement of cryopreservation protocols (Nasrin *et al.* 2012).

Transaminases are located primarily in the mid-piece of spermatozoa (Mann, 1964), whereas, LDH is localized in cytosol and mitochondria of spermatozoa (Burgos *et al.* 1995). Enzymes are used as good indicators of semen quality because they measure plasma membrane stability of spermatozoa (Sirat *et al.* 1996). The presence of certain enzyme activities indicate reproductive seasonality (Mustaffa, 2005) and the possible source of these enzymes in seminal plasma is thought to be the testes or epididymides (Kareskoski and Katila, 2008).

Since there was paucity of information on the enzymatic activities of seminal plasma of Nari Suvarna rams, the activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were determined in the present study. Also, after refrigerating the semen for 24 hrs at 4°C in Tris and citrate buffer, the activities of these enzymes were estimated in order to know the extent of enzyme leakage from the spermatozoa in to the seminal fluid.

Materials and Methods

Collection of semen and processing for seminal plasma

Six healthy and sexually matured Nari Suvarna rams having good body configuration, well-developed testes and good vigor within the age group of two to three years which were having good libido and adaptability to artificial

vagina semen collection technique were selected for the present study. A total of 16 ejaculates from each ram, during eight weeks time at the rate of two collections per week were collected during the study period from January to February, 2015.

Immediately after collection, the semen was transferred to Eppendorf tubes of one ml capacity and transported to the laboratory by keeping in thermos flask in which water temperature was maintained at 34°C.

The Eppendorf tubes with the semen samples were centrifuged in temperature regulated centrifuge at 34°C at 5000 rpm for 15 minutes. Separated seminal fluid from each tube was aspirated carefully using micropipette and transferred in to individual sterile Eppendorf tubes and the enzymes were determined.

Determination of enzyme activities in fresh seminal plasma

The activities of ALP, ALT and AST were estimated using reagent kits, which are based on the procedures such as Tietz (1986), Bardley *et al.* (1972) and Tietz (1986), respectively, manufactured by Transasian Bio-Medicals Ltd., Himachal Pradesh, India with the help of ERBA biochemical analyzer. LDH activity was estimated using the reagent kits manufactured by Euro Diagnostic systems Pvt. Ltd. Chennai, India which is based on the method of Tietz (1986) with the help of ERBA biochemical analyzer.

Extension of semen, refrigeration for 24 hrs and determination of enzyme activities

The pooled semen sample from four rams was extended with Tris-egg yolk based and sodium citrate egg yolk based extenders by adjusting the concentration of sperms to 200 million per ml. The enzyme activities were estimated immediately after the extension of semen and the values were considered as before refrigeration. The extended semen samples were kept for 24 hrs refrigeration at 4°C and then subjected to centrifugation to obtain the seminal plasma. The activities of enzymes, *viz.*, ALP, ALT, AST and LDH were estimated immediately as per the procedure followed for the fresh samples.

Statistical analysis

The data was analyzed with the aid of statistical software, Graph Pad Prism version 5.01 by application of paired t-test and the significance was determined at $P < 0.05$.

Results and Discussion

Enzyme Activities in Fresh Seminal Plasma

There was non-significant difference ($P>0.05$) in the activities of ALT, AST and LDH in the seminal plasma between individual rams. The ALP activity was significantly lower only in ram number 6 compared to all other rams (Table 1).

Table 1: Mean \pm SE values of enzymatic activity (IU/L) of seminal plasma of Nari Suvarna rams

Ram number	ALP	ALT	AST	LDH
1	740.25 \pm 6.00 ^a	46.10 \pm 4.39	46.15 \pm 3.75	562.00 \pm 32.59
2	720.63 \pm 6.00 ^a	52.08 \pm 4.91	38.75 \pm 4.67	594.38 \pm 29.55
3	717.75 \pm 7.56 ^a	44.42 \pm 5.94	37.96 \pm 5.18	595.75 \pm 26.04
4	732.63 \pm 4.73 ^a	50.29 \pm 4.05	39.25 \pm 4.20	649.63 \pm 32.04
5	744.75 \pm 4.20 ^a	44.69 \pm 6.41	43.15 \pm 4.82	528.63 \pm 19.48
6	706.75 \pm 5.37 ^b	32.11 \pm 2.13	40.87 \pm 5.13	628.63 \pm 34.55
Mean \pm SE values	724.50 \pm 2.96	44.95 \pm 4.40	41.02 \pm 1.84	593.17 \pm 12.78

Seminal ALP activity is observed on sperm head, mid-piece and tail fragments. It is known to regulate phosphorylation of proteins by the cAMP-dependent protein kinase pathway which is necessary for spermatozoa motility. ALP leakage in to the seminal plasma from the spermatozoa could be used as a marker for optimization of cooling and freeze thaw steps for cryopreservation of ram semen (Upreti *et al.* 1996). The alkaline phosphatase activity of the present observation in the Nari Suvarna ram semen corroborated with the findings of Rai *et al.* (1976) in Nali ram and Bharti *et al.* (2010) in Chottanagpuri rams.

Significantly higher ALP enzyme activity observed in a ram compared to other rams in the present study was in agreement with the findings of Mandal (1990) in Black Bengal bucks and Kale and Tomar (2000) in crossbred (alpine \times Beetal, Saanen \times Beetal) bucks semen and one of the reason could be aging and decaying spermatozoa in ejaculates.

The non significant ($P>0.05$) differences of ALT and AST activities in the seminal plasma of individual Nari Suvarna rams in the present study were in accordance with the observations of Mandal (1990) in Black Bengal buck and Kale and Tomar (2000) in crossbred bucks (Alpline \times Beetal and Saanen \times Beetal) and Bharti *et al.* (2010) in Chottanagpuri rams.

The activity of transaminase enzymes such as ALT and AST in semen is a good indicator of fertility because it measures sperm membrane stability (Pace and Graham, 1970).

The present findings of non significant variation in LDH activity among Nari Suvarna rams was in agreement with the findings of Bharti *et al.* (2010) in the semen of Chottanagapuri rams. However, the observed values of LDH activity in the present study were higher than those reported in Konya Merino rams by Kaya *et al.* (2002), in Iranian Moghani rams by Zamiri *et al.* (2010) and in Baluchi × Moghani, Ghezel × Baluchi, Ghezel × Merino and Merino × Moghani rams by Asadpour (2012). The LDH enzyme could be responsible for driving glycolysis when oxygen is limited by carrying NADH-mediated reduction of pyruvate to lactate, and reduced LDH activity in seminal plasma indicate disturbed spermatozoal function and metabolism (Jones, 1997).

Table 2: Mean ± SE values of enzymatic activity (IU/L) before and after refrigeration of extended semen with Tris and sodium citrate buffers

Enzymes	Before refrigeration with extenders	After refrigeration with Tris buffer	After refrigeration with citrate buffer
ALP	743.25 ± 25.41	796.75 ± 52.17	765.00 ± 30.21
ALT	58.25 ± 3.01	65.89 ± 1.78	63.77 ± 2.10
AST	35.96 ± 8.86	53.04 ± 15.47	53.04 ± 14.02
LDH	662.50 ± 41.31	695.25 ± 35.60	686.25 ± 39.86

Enzyme activities in the extended and refrigerated semen

Numerically increased activities of ALP, ALT, AST and LDH enzymes after refrigeration were observed when compared to the state of before refrigeration both in Tris and citrate buffer extended semen (Table 2).

The increase in activity of these enzymes was in accordance with the findings of Belorkar *et al.* (1988) and Stefanov *et al.* (2013) in boar semen. This was probably due to damage to sperm membrane and subsequent leakage of enzymes in to extracellular fluid due to cold shock (Jani *et al.* 1983; Dhami and Kodagali, 1987).

It was concluded that the enzymatic activities such as ALT, AST and LDH in the seminal plasma did not vary significantly between Nari Suvarna rams except higher ALP activity in one ram. The increased enzymatic activities of ALP, ALT, AST and LDH after refrigeration for 24 hrs at 4°C could be due to leakage of these enzymes during the storage period, indicating that the damage to plasma membrane of spermatozoa has occurred due to cold shock.

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