

**Effect of Tris and Biociphos-Plus Extenders on the Extracellular Enzyme Release of Phosphatases and Transferases in Punganur Bull Semen**K. Veerabramhaiah<sup>1\*</sup>, A. Seshagiri Rao<sup>1</sup>, V.H. Rao<sup>2</sup>, K. Venugopal Naidu<sup>1</sup>, S.T. Viroji Rao<sup>3</sup><sup>1</sup>Department of Gynaecology, College of Veterinary Science, Tirupati – 517502, A.P., India<sup>2</sup>Department of Veterinary Physiology, CVSc, Tirupati, A.P., India<sup>3</sup>AICRP on Poultry, Rajendranagar, Hyderabad, A.P., India

(Received 4 May 2011/ Accepted 2 Juli 2011)

**Abstract**

The present investigation was carried out to study phosphatase and transferase enzymes leakage in the neat and extended Punganur bull semen before and after freezing with Tris and Biociphos-plus extenders. Among the bulls studied, the Alkaline Phosphatase (AKP) concentration in fresh semen was significantly different but Acid Phosphatase (ACP) was not significantly different and a positive correlation between the concentration of AKP and semen volume, individual motility, sperm concentration and total abnormalities were observed. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) concentrations were not significantly different and positively correlated with individual motility, sperm concentration, percentage of live sperms and acrosomal damage. Leakage of AKP, ACP, AST and ALT enzymes before freezing the semen of Punganur bulls was observed to be significantly higher in the semen extended with Biociphos-plus extender.

*Keywords:* Punganur bulls; Semen; Phosphatases; Transferases; Tris buffer; Biociphos-plus

**Introduction**

Punganur cattle the world's shortest humped cattle (*Bos indicus*) with long tail and switch touching the ground are at the risk of extinction (Ramesha, 2001). To prevent further reduction in the number of this endangered cattle in addition to the existing in-situ conservation method (Smith, 1984) ex-situ conservation method was taken up through cryopreservation of male germplasm (Balain, 1989). Hence, it was proposed to take up the present study to preserve and assess the fertility of the semen by pre and post thaw enzyme leakages by using conventional and commercial semen extensors.

**Materials and methods**

Experiments were designed to conserve the Punganur cattle germplasm and to estimate the enzymes in seminal plasma immediately after collection and

before and after cryopreservation at Livestock Research Station, Palamaner, Chittoor Dist, A.P and Department of Animal Reproduction and Gynaecology, College of Veterinary Science, Tirupati.

Semen from ten bulls aged between 6 to 10 years was utilized at 20 ejaculates per each bull twice in a week. During each collection  $\frac{1}{4}$  to  $\frac{1}{2}$  of the ejaculates having more than 60 percent individual motility were analyzed for the estimation of ACP, AKP, AST and ALT and keeping quality of semen in two extenders i.e., Tris extender and Biociphos-plus extender based on estimation of extra-cellular leakage of enzymes in the extended semen before and after freezing.

Tris extender is routinely used extender for bull semen containing Tris (Hydroxy methyl aminonethane) 2.42 g; Citric acid monohydrate 1.36 g; Fructose 1.00 g; Glycerol 6.4 ml; Egg yolk 20.0 ml and Double distilled water added up to 100.0 ml. Before addition of Tris extender, semen samples were divided into two equal split samples immediately after collection and to one split sample was diluted with Tris extender at 1:3 ratio (Foote, 1970). Another split sample was extended with Biociphos-plus extender as detailed below.

\*Corresponding author: K. Veerabramhaiah

Address: Department of Gynaecology, College of Veterinary Science, Tirupati – 517502, A.P., India

E-mail address: bramhaiahvet@yahoo.com

The Biociphos-plus is a readymade extender manufactured and supplied by M/s IMV L'Aigle, France having saline solution, egg yolk substitute, glycerol and antibiotics (Gentamycin, Tylosin, Lincomycin and Sp actinomycin). The exact concentration of each of these constituents was not furnished by the manufacturer. The final solution of Biociphos-plus was prepared for 500ml final solution by following manufacturers' directions (Thun *et al.*, 2002). Equal volume of semen and Biociphos-plus were mixed based on the concentration of the semen and kept in a water bath for 10 minutes. Finally, it was brought to room temperature by placing on a lab table for 15-20 minutes. Care was taken to maintain desired concentration of 30-40 millions spermatozoa per dose. At the end of equilibration, semen samples were collected for assessing the pre freeze semen characteristics from each ejaculate. Then the freezing process was performed by following standard rapid freezing procedure for both the extenders. After 24 hours of freezing, straws from each batch of cryopreserved semen diluted with Tris and Biociphos-plus were thawed in distilled water having 37°C temperature for 30 sec and the seminal plasma was obtained by centrifuging thawed semen at 2000 rpm. Then the seminal plasma was used for estimating leakages of enzymes. All these enzyme levels in neat and diluted seminal plasma were estimated by using commercially available Diagnostic kits (Span Diagnostics Limited, 173-B, New Industrial Estate, Udhna, SURAT, India).

#### Statistical analysis

The data was analyzed by using SPSS 12.0 for win-

Table 1. Enzyme levels in seminal plasma of Punganur bull semen extended with Tris or Biociphos-plus extenders (Bulls (10) x Ejaculates (20) n=200)

		AKP (KAU/100 ml)	ACP (KAU/100 ml)	AST (IU/ml)	ALT (IU/ml)
Neat semen		583.43±0.71	241.12±0.18	14.56±0.19	49.24±0.82
Tris Extender	Pre-freeze	15.58±0.17a	9.23±0.13a	11.68±0.12a	38.82±0.64
	Post-thaw	17.07±0.17a	10.72±0.14a	13.17±0.17a	42.99±0.65
Biociphos plus extender	Pre-freeze	18.66±0.26b	11.87±0.14b	13.00±0.11b	38.53±0.96
	Post-thaw	20.32±0.26b	13.53±0.15b	14.67±0.17b	42.98±0.96

Data expressed as mean±S.E.

Values bearing different superscripts (a, b) within a column differ significantly ( $P \leq 0.05$ )

dows (analysis of variance (two way classification)) at  $P < 0.05$  level significance.

#### Results

The overall mean activities of AKP (KAU/100ml), ACP (KAU/100ml), AST (IU/ml) and ALT (IU/ml) enzymes in the fresh seminal plasma of Punganur bulls observed in the present study were 583.43±0.71, 241.12±0.18, 14.56±0.19 and 49.24±0.82, respectively. AKP concentration differed significantly among the bulls but ACP, AST and ALT did not (Table 1).

Leakage of AKP, ACP, AST and ALT enzymes before freezing in semen extended with Tris extender were 15.58±0.17, 9.23±0.13, 11.68±0.12 and in semen extended with Biociphos-plus extender were 38.82±0.64 and 18.66±0.26, 11.87±0.14, 13.00±0.11 and 38.53 ± 0.96, respectively. While the same during post thaw period in semen extended with Tris extender were 17.07±0.17, 20.32 ± 0.26, 13.17±0.17 and 42.99±0.65 and in semen extended with Biociphos-plus extender were 10.72±0.14, 13.53±0.15, 14.67± 0.17 and 98±0.96, respectively (Table 1).

The pre freeze and post thaw leakages of AKP, ACP and AST in Biociphos-plus extended semen were significantly higher than the leakages in Tris extended semen but leakage of enzyme ALT was insignificantly higher (Table 1). There was a positive correlation between AKP and semen volume, individual motility, sperm concentration and total abnormalities; between ACP and sperm concentration; between AST and mass activity, individual motility, sperm concentration, percentage of live sperms and acrosomal damage and between ALT

and semen volume, individual motility, sperm concentration, percentage of live sperms and acrosomal damage.

## Discussion

The AKP and ACP are the two most active dephosphorylation enzymes present in semen, which is directly related to the fertility. Their concentrations reflect the functional status of the accessory sex glands and metabolic activity of spermatozoa. The overall mean activity of AKP in the fresh seminal plasma of Punganur bulls observed in this investigation was slightly higher than the present study in Punganur bulls (Baburao, 1996). The present observation is in agreement with the reports of Swain and Singh (2004) in Sahiwal bull and Dhama and Kodagali (1988) in buffalo bulls. Lower concentrations of AKP were observed by Srivastva and Satish kumar (2004) in Holstein Friesian bulls but same were not comparable with the observations in crossbred bulls (Mishra *et al.*, 1969 and Nadroo *et al.*, 1987) and in Argentine bulls (Aguirre *et al.*, 1988) as they have reported the concentrations of AKP in different units. The overall mean activity of ACP in the fresh seminal plasma observed in this investigation was similar to the findings of Baburao (1996) in Punganur bulls. These concentrations are also comparable with the reports in crossbred bulls by Mishra *et al.* (1969), but higher values were reported in Argentine (Aguirre *et al.*, 1988), crossbred (Srivastva and Satish kumar, 2004) and Sahiwal bulls (Swain and Singh, 2004). The activity of AKP was higher than ACP in this study, which is in agreement with Reid *et al.* (1948).

The concentration of ALT in seminal plasma must be high for semen with good freezability (Prasad *et al.*, 2000). No work was undertaken earlier to estimate the concentration of ALT and AST in Punganur bull semen. However, concentration of ALT was higher and AST was comparable to the present study in Sahiwal bulls (Swain and Singh, 2004). Among the Punganur bulls studied, the ALT and AST concentrations were not significantly different and were positively correlated with individual motility, sperm concentration, percentage of live sperms and acrosomal damage. These findings are in line with the observations of Patel *et al.* (1989). Present study ALT and AST concentrations in seminal plasma are indicating good fertilizing capacity of Punganur bull semen (Pangawkar *et al.*,

1989; Verma, 1999 and Swain and Singh, 2004).

Until the date, no attempt was made to estimate extra-cellular leakage of enzymes in the extended semen of Punganur bull. Leakage of AKP, ACP, ALT and AST enzymes before freezing the semen of Punganur bulls was observed to be significantly higher in the semen extended with Biociphos-plus extender. Dhama and Kodagali (1988) also reported a significant effect of extenders on the leakage of AKP. Diluting the semen sometimes causes dilution effect (Harrison and White, 1972) leading to the release of intra cellular proteins and enzymes from spermatozoa. This probably causes damage and increases membrane permeability (Vyawanare *et al.*, 1989). Higher leakage of all these enzymes in the semen extended with Biociphos extender than the Tris could be attributed to the protective action of Tris on sperm membrane against cellular injury (Tuli *et al.*, 1982 and Vyawanare *et al.*, 1989).

The post thaw leakage of AKP, ACP, ALT and AST enzymes in the present study are higher than the observations of Verma *et al.* (1999) in half bred Holstein-Friesian x Harijana bulls but lower than the observations of Belorkar *et al.* (1987), Sundararaman *et al.* (1997), Prasad *et al.* (2000) and Srivastva and Satish kumar (2004) in crossbred bulls. Leakage of AKP, ACP and ALT after freezing and thawing of Punganur bull's semen extended with Biociphos-plus extender was significantly higher, but leakage of the enzyme AST was not significant. Usually leakage of enzymes from sperm occurs, if there is any damage to the sperm membrane due to cold shock or unfavorable concentration of extender ingredients (Singh *et al.*, 1992) and estimation enzymes in turn reflect the efficacy of the extender in maintaining the membrane integrity of spermatozoa during freezing and thawing (Prasad *et al.*, 2000). In general the best semen extender can block the leakage of enzymes and other electrolytes from the sperm cell. In the present study enzyme leakages were lesser in the semen extended with Tris than Biociphos-plus extender. Considering these factors it may be concluded that Tris extender may be a better choice for preservation of Punganur bull semen.

## References

- Aguirre, S.M., Capaul, E.G., Luca, L.D., Cinque, M., 1988. Total and non-prostatic acid phosphatase and alkaline phosphatase activity in bull and ram semen. *Verterinaria Argentina* 5,

282-287.

- Baburao, k., 1996. Studies on semen characteristics and keeping quality of semen in Punganur bulls. M.V.Sc. Thesis. Andhra Pradesh Agricultural University, Hyderabad.
- Balain, D.S., 1989. Salient aspects of conservation, evaluation and management of animal genetics resources. Silver Jubilee Celebrations Veterinary College, Anand, Lead Papers and Abstracts, National Seminar on genetics applied to livestock production, pp. A126-A134.
- Belorkar, P.M., Dhama, A.J., Kodagali., 1987. Levels of transaminases and phosphatases in relation to freezability and fertility in crossbred bulls. *Artificial Insemination New* 2, 1-4.
- Dhama, A.J., Kodagalli, S.B., 1988. Leakage of phosphatases from buffalo bulls spermatozoa during freezing - Effect of dilutors, seasons, bulls and stages of freezing. *Indian Journal of Animal Reproduction* 9, 124-131.
- Foote, R.H., 1970. Fertility of Bull Semen at High Extension Rates in Tris-Buffered Extenders. *Journal of Dairy Science* 53, 1475-1477.
- Harrison, R.A.P., White, I.G., 1972. Glycolytic enzymes in the spermatozoa and cytoplasmic droplets of bull, boar and ram, and their leakage after shock. *Journal of Reproduction and Fertility* 30, 105-115.
- Mishra, B.S., Singh, B.P., Tomar, N.S., 1969. Studies on biochemical attributes of buffalo semen. *Indian Journal of Dairy Science* 22, 202-203.
- Pangawkar, G.R., Sharma, R., 1989. Spermiorgam and cytomorphology of spermatozoa in relation to freezability of Holstein-Friesian semen. *Indian Journal of Animal Reproduction* 10, 60-63.
- Patel, K.V., Dhama, A.J., Kodagali, S.B., 1989. Seminal characteristics and their relationship in Kankrej X Jersey HF crossbred bulls. *Indian Veterinary Journal* 66, 737-743.
- Prasad, J.K., Satish Kumar, Mohan Greesh, Agarwal, S.K., Uma Shanker, 2000. Biochemical studies pertaining to freezability of cross bred bull semen. *Indian Journal of Veterinary Research* 9, 37-40.
- Ramesha, K.P., 2001. Commissioned paper in the Thematic Working Group on Domesticated Bio- Diversity, National Bio-diversity Strategy and Action Plan, Ministry of Environment and Forestry, Government of India.
- Reid, J.T., Ward, G.M., Salisbury, R.L., 1948. Effects of certain factors upon the level of the plasma phosphatases of breeding bulls. *American Journal of Physiology* 153, 225.
- Singh, R.S., Tomar, N.S., Sharma, K.C., Sharma, K.B., 1992. Studies on acrosomal abnormalities of cattle and buffalo spermatozoa in relation to other semen characteristics and fertility. *Indian Veterinary Journal* 69, 267-268.
- Smith, C., 1984. Estimated cost of genetic conservation in farm animals. *FAO Animal Production and Health Paper* 44, 21-30.
- Srivastava, S., Satish Kumar, 2004. Effect of additives on enzyme leakage during cryopreservation of bull semen. *Indian Journal of Animal Sciences* 74, 918-921.
- Sundaraman, M.N., Thangaraju, P., Edwin, M.J., 1997. Effect of deep freezing on intracellular enzyme leakage in Jersey bull semen. *Cheiron* 26, 60-62.
- Swain, A.K., Singh., 2004. Enzymatic profile in relation to physical parameters of Sahiwal bull semen. *Indian Journal of Animal Sciences* 74, 594-596.
- Thun, R., Hurtado, M., Janett, F., 2002. Comparison of Biociphosplus and TRIS-egg yolk extender for cryopreservation of bull semen. *Theriogenology* 57, 1087-1094.
- Tuli, R.K., Singh, M., Matharoo, J.S., 1982. *Animal breeding Abstracts*, 51: 879 cited by Vyawanare, R., Chauhan, R.A.S., Nema, S.P., Porwal, M.L., 1989. Studies on seminal attributes, enzyme leakage and preservability of buffalo semen. *Indian Veterinary Journal* 66, 1128-1132.
- Verma, N.K., Satish Kumar, Mohan, G., Bisht, G., 1999. Freezability and enzyme leakage of crossbred bull semen in the dilutors in presence of chlorpomazine HCL. *Indian Journal of Animal Sciences* 69, 770-772.
- Vyawanare, R., Chauhan, R.A.S., Nema, S.P., Porwal, M.L., 1989. Studies on seminal attributes, enzyme leakage and preservability of buffalo semen. *Indian Veterinary Journal* 66, 1128-1132.