EFFICACY OF THE TRIS AND BIOCIPHOS PLUS EXTENDERS ON THE FREEZABILITY OF PUNGANUR BULL SEMEN

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ABSTRACT

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The present investigation was carried out to study the freezability of Punganur bull semen with Tris and Biociphos plus extenders. The difference in the individual sperm motility and live sperms between fresh and Biociphos plus extended semen and between Tris and Biociphos plus extended semen during pre-freeze and post-thaw periods was significant. The difference in head, mid- piece, tail and total sperm abnormalities between fresh and Tris extended semen was not significantly different but between Tris and Biociphos plus extended semen during pre-freeze and post-thaw periods was significant. Both the extenders were relatively non-toxic to sperm, but based on the sperm motility, livability, sperm abnormalities and acrosome damage Tris extender was concluded a better choice for preservation of Punganur bull semen.

Key words : Punganur bulls, Semen, Freezability, Tris buffer, Biociphos Plus

INTRODUCTION

Punganur the world's shortest humped cattle (*Bos indicus*), with long tail and switch touching the ground is at the risk of extinction (Ramesha, 2001). Conservation of endangered breed would prevent further reduction of current population by implementing carefully planned mating programmes in conjunction with appropriate reproductive technologies. Hence, it was proposed to take up the present study in order to preserve semen and assess pre-freeze and post-thaw quality by using conventional and commercial semen extenders.

MATERIALS AND METHODS

The present study was undertaken at the Livestock Research Station, Palamaner, Chittoor Dist, Andhra Pradesh and Department of Animal Reproduction, Gynaecology & Obstetrics, College of Veterinary Science, Tirupati. Experiments were designed to conserve the Punganur cattle germplasm and to study semen characteristics immediately after collection, before and after cryopreservation.

Ten Punganur bulls aged between 6 and 10 years and maintained in semi-intensive housing system were utilized for the study. A total of 20 ejaculates from each bull, twice in a week, were collected and analyzed for various semen characteristics like volume (ml), mass activity (0-4 scale), individual motility (%), sperm concentration (millions/ml), live sperms (%), acrosomal damage (%),sperm head abnormalities (%),mid piece abnormalities (%), tail abnormalities (%) and total sperm abnormalities. The ¼ to ½ of each ejaculate having more than 60 percent individual sperm motility was utilized to study the progressive sperm motility, morphology and acrosomal damage before and after cryopreservation in Tris and Biociphos plus extenders.

Immediately after evaluation, some portion of semen was extended with Tris extender as per the standard procedure of Foote (1970) and the remaining portion with the Biociphos plus of M/s IMV L'Aigle, France as per manufacturers' procedure. Care was taken

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to maintain desired concentration of 10 million live spermatozoa per dose. Semen samples were taken at the end of equilibration for assessing pre-freeze characteristics. Later freezing process was performed by following standard rapid freezing protocol for both the extenders and after 24 hrs of freezing one straw from each batch of cryopreserved semen diluted with Tris and Biociphos plus was thawed at 37°C for 30 sec and used for analyzing post-thaw characteristics. The data was analyzed by using SPSS 12.0 for windows (analysis of variance (two way classification)) at $P<0.05$ level significance.

RESULTS AND DISCUSSION

As per the FAO norms (Anon, 1990) Punganur breed is in an alarming state of extinction. Extensive crossbreeding with exotic dairy breeds like Jersey, Holstein Friesian and Kerry in particular (Annual report of LRS, Palamaner, 1994-95) has reduced the number of pure Punganur cattle to just about 70 (Pundir and Sahai, 1997) in its home tract. As an adjunct to the ongoing in situ conservation of Punganur cattle germ plasm, ex-situ conservation of semen was attempted in this study.

The overall mean individual sperm motility (%) and live sperms (%) in fresh semen of Punganur bulls were significantly different among the bulls, but the head, mid piece, tail and total abnormalities of sperm did not differ significantly among the bulls. Similar observations on semen characteristics were recorded by Baburao (1996) in Punganur bulls.

Since, no work has been carried out earlier to this study, to evaluate pre-freeze and post-thaw semen characteristics in Punganur cattle, suitability of Tris and Biociphos plus extenders for dilution and freezing of Punganur bull semen was assessed (Table).

The difference in the percentage of motile and live sperms between the fresh and Tris extended semen was not significant, but between fresh and Biociphos plus extended semen was significant. This indicates that Tris has supported the dilution of Punganur semen well than Biociphos plus extender which might be due to reduced motility and livability of sperm after addition of Biociphos plus during the process of dilution, glycerolisation and equilibration of semen (Sharma *et al.*, 1992) (Table).

Significant difference observed between Tris and Biociphos plus diluted semen in the pre-freeze motility and livability (Table 1) is in accordance with reports of Leeuw and Haring (2000) and Thun *et al.* (2002), but contradicts the finding of Moussa *et al.* (2002) and Amirat *et al.* (2004). Higher sperm motility and livability of sperm in Tris might be due to its better buffering capacity and penetration of Tris in to sperm cells (Dhami and Sahni, 1994). Lesser motility and livability in Biociphos plus extender may be ascribed to the lowered osmotic resistance of spermatozoa in the presence of Biociphos plus (Thun *et al.*, 2002).

Sharma *et al.* (1992) recorded slightly higher acrosomal damage than the present value. The difference in acrosomal damage between the fresh and extended semen with both the extenders and also between Tris and Biociphos plus extended semen was significant (Table). This could be due to the differential response of the sperm cell membrane to the extenders used and some primary defects in spermatozoa (Sharma *et al.*, 1992).

The percentages of pre-freeze sperm head, mid piece, tail and total abnormalities of Punganur semen extended with Tris extender and Biociphos plus were not significantly different from the values observed in fresh semen, although significant differences were noticed between extenders (Table). It may be ascribed to genetic makeup of bulls, inherent differences in the spermatozoa and premature release of hydrolytic enzymes which in turn causes mid piece abnormalities (Barth and Oko, 1989). It may also be due to better penetration of Tris in to sperm cells (Dhami and Sahni, 1994).

The percentages of post-thaw sperm motiiity and livability of semen extended with Tris or Biociphos plus were significantly lower than the values observed in the pre-freeze semen (Table) but their percentages are well within the normal range for obtaining the good fertility.

This might be due to the process of freezing where one third of the cells are killed and rendered non functional on account of various factors (Pickett *et al.*, 1965). Thus both Tris and Biociphos plus appeared to have protected the sperm well during freezing and/or thawing.

Post-thaw individual motility and livability of sperms in Punganur bulls was significantly different between Tris and Biociphos plus extended semen (Table). Thus Biociphos plus extender was less effective at protecting spermatozoa during freezing and thawing (Burgel, 2001 and Thun *et al.*, 2002).

The percentages of post-thaw acrosomal damage of Punganur semen extended with Tris or Biociphos plus were significantly increased as compared to the values observed in the pre-freeze semen. The difference in the post-thaw acrosomal damage between extenders was also significant (Table). The differences in the acrosomal damage between pre-freeze and post-thaw semen may be due to the effect of freezing and difference in cryoprotective capabilities of extenders.

The percentages of post-thaw sperm head, mid piece, tail and total abnormalities of Punganur semen extended with Tris or Biociphos plus were not significantly different from the values observed in the pre-freeze semen. But significant differences were noticed between Tris and Biociphos plus extended semen with regard to post-thaw head, mid piece, tail and total sperm abnormalities in Punganur bulls (Table). High percentage of post-thaw sperm abnormalities with Biociphos plus were also reported by Hurtado *et al.* (1997) and Bozkurt and Tekin (2002) in Holstein Friesian bulls. Layers of frozen Biociphos plus extender were also too tiny to protect all spermatozoa from contact with large ice-crystals (Amirat *et al.*, 2005) during freezing.

In the present study, both the extenders were relatively non-toxic to cells but the percentages of reduction in sperm motility and livability and increase in acrosome damage and plasma membrane alterations in Tris were lesser than Biociphos plus extended semen and hence it was concluded that Tris extender may be a better choice for preservation of Punganur bull semen.

TABLE : SEMEN CHARACTERISTICS OF PUNGANUR BULL SEMEN EXTENDED WITH TRIS OR BIOCIPHOS PLUS EXTENDERS (BULLS (10) X EJACULATES (20) N=200)

Extender		Individual motility	Live sperm	Abnormalities				
				Head	Acro-somal	Mid piece	Tail	Total
Fresh semen		72.85 ± 0.71^{A}	79.34 ± 0.33^{A}	4.25 ± 0.07^{A}	4.75 ± 0.07^{A}	1.97 ± 0.27^{A}	4.25 ± 0.08^{A}	10.46 ± 0.12^{A}
Pre-freeze	Tris extender	70.08 ± 0.39^{aA}	76.33 ± 0.33^{aA}	5.25 ± 0.07^{aA}	20.84 ± 0.37^{aA}	2.97 ± 0.06^{aA}	5.25 ± .08^{aA}	11.46 ± 0.12^{aA}
	Biociphos plus extender	67.55 ± 0.40^{bB}	71.34 ± 0.33^{bB}	7.59 ± 0.08^{bB}	23.30 ± 0.39^{bB}	5.27 ± 0.07^{bB}	7.55 ± 0.17^{bB}	13.46 ± 0. 12^{bB}
Post-thaw	Tris extender	55.33 ± 0.95^{a}	61.59 ± 0.82^{a}	5.90 ± 0.15^{a}	25.19 ± 0.39^{a}	3.61 ± 0.13^{a}	5.90 ± 0.18^{a}	12.10 ± 0.19^{a}
	Biociphos plus extender	47.55 ± 0.65^{b}	51.33 ± 0.33^{b}	8.69 ± 0.11^{b}	28.26 ± 0.43^{b}	6.37 ± 0.13^{b}	8.65 ± 0.17^{b}	14.56 ± 0.12^{b}

Values bearing different superscripts (A, B or a, b) within a column differ significantly (Pd"0.05)

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