

## Post thaw evaluation and fertility of bovine semen diluted in Biociphos plus and tris extenders\*

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### ABSTRACT

A comparative study on post thaw semen characteristics and fertility of Murrah and Jersey bull semen diluted with a new yolk free "Biociphos plus" and routinely used yolk containing "Tris" extenders was undertaken. The variation in overall prefreeze motility was found to be significant ( $P < 0.05$ ) between the two diluents in both the breeds. Also significant difference was noticed in the post thaw motility ( $P < 0.01$ ) and post thaw acrosomal damage ( $P < 0.05$ ). Interestingly, Biociphos plus was found to be highly superior to Tris in arresting bacterial contamination of semen. However, the fertility rates between diluents did not differ much in both the breeds.

**Key words :** Biociphos plus, tris, semen extenders, post thaw motility of spermatozoa, fertility, bovine semen

### INTRODUCTION

It is essential to select an appropriate extender to retain the functional integrity of bovine spermatozoa for cryopreservation (Hinsch *et al.*, 1997). Routinely used extenders contain upto 20 per cent egg yolk and hence higher the risk of bacterial contamination (Heydron and Paufler, 1973 and Ahmed and Foote, 1986). Presence of abnormal microflora in the semen beyond certain levels may reduce the survivability of spermatozoa and fertility of cows (Gupta and Maurya., 1993). The present study was, therefore, planned to compare various post thaw semen characteristics including bacterial load and fertility rate after freezing/ thawing between Biociphos plus, an yolk free diluent and Tris, the yolk containing diluent in Murrah and Jersey bulls.

### MATERIALS AND METHODS

Six bulls each from Jersey and Murrah aged between 3 and 11 years and maintained under uniform management conditions were used for the present study. All the bulls are routinely used for semen collection twice a week by AV method using a male dummy. A total of 72 ejaculates (36 each from Murrah and Jersey

breed) were analyzed by standard methods (Suryaprakasam T B and Rao AVN 1993) for various semen characteristics such as volume, mass activity, initial motility, sperm concentration, live sperm count, percent sperm abnormalities and acrosomal damage. Following initial evaluation each fresh ejaculate from both the breeds was split equally, diluted with Tris and Biociphos plus separately and equilibrated for six hours at 5 °C. At the end of equilibration, a drop of diluted semen from both the diluents was used for assessing pre freeze motility. The diluted semen was packed in 0.25 ml French mini straws.

The ramps containing straws were placed in the Biofreezer (Digit cool IMV, France) at 5 °C. The temperature is reduced @ -4 °C / minute from 50°C to -100°C and @ -400°C /minute from -100°C to -150°C. The straws were then plunged into liquid nitrogen at -196°C. 24 hrs after freezing, one straw from each batch of semen was thawed and the efficacy of Tris and Biociphos plus dilutors was evaluated based on post thaw motility, live spermcount, total sperm abnormalities, acrosomal damage, bacterial load and fertility rate. The data was analyzed by adopting the methods described by Snedecor and Cochran (1967).

### RESULTS AND DISCUSSION

The results of pre freeze motility and post thaw semen characteristics in relation to two diluents sin

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**Table 1. Pre freeze motility and post thaw semen characteristic between Biociphos plus and Tris diluents in Murrah and Jersey bulls**

Character	Biociphos plus		Tris	
	Murrah	Jersey	Murrah	Jersey
Prefreeze motility (%)	63.88±0.98	65.83±1.02	60.27±1.14	61.67±0.91
Post thaw motility (%)	55.00±1.10	55.83±0.92	49.16±0.86	48.33±0.71
Post thaw live sperm (%)	57.91±0.76	57.43±0.65	55.75±0.83	54.95±0.60
Post thaw total sperm abnormalities (%)	16.83±0.41	15.66±0.39	17.33±0.42	16.75±0.37
Post thaw acrosomal damage (%)	37.66±0.55	36.33±0.69	39.83±0.67	38.15±0.44
Bacterial count (number of colonies per ml of semen)	46.66±19.08	33.33±12.29	1213.33±89.24	1146.60±69.79
Fertility (number pregnant for 100 AI)	45.33±2.43	51.00±2.48	40.33±2.70	44.67±2.67

Murrah and Jersey breeds are presented in the Table 1.

The pre freeze motility was found to be significantly higher in Biociphos plus diluted semen in both the breeds. Due to absence of egg yolk the Biociphos plus diluent provided more clarity. A highly significant variation ( $P<0.01$ ) was observed between the two diluents in both the breeds with regard to post thaw motility. The variation in post thaw live sperm count between the diluents was significant ( $P<0.05$ ) in Murrah breed and highly significant ( $P<0.01$ ) in Jersey breed. The post thaw total sperm abnormalities in the present study did not differ significantly between Tris and Biociphos plus in Murrah bulls. However, in Jersey bulls a significant difference ( $P<0.05$ ) was observed between the two diluents. The post thaw acrosomal damage was observed to be significantly lower in Biociphos plus diluted semen compared to that of Tris. In contrast to the present observation a high acrosomal damage of 52.00 (Hinsch *et al.* 1997) and 86.00 per cent (Bohm *et al.*, 1996) was reported with Biociphos plus dilutor in exotic cattle.

Bacterial growth was significantly arrested in Biociphos plus diluted semen with only  $46.66 \pm 19.08$  and  $33.33 \pm 12.29$  colonies per ml. of semen in Murrah and Jersey bulls respectively. Due to absence of yolk protein and the presence of higher antibiotics like Gentamycin, Tylosine, Linomycin and spectinomycin in Biociphos plus the bacterial growth might have been arrested to greater extent. The fertility rates obtained with Biociphos plus and Tris diluent in Murrah bulls are comparable with the conception rate of 44.69 per cent

reported by Bhavasar *et al.* (1986). However, Raizada *et al.* (1990) reported a higher conception rate of 60 per cent in Tris diluted Murrah semen. Also conception rate of 44.67 per cent obtained in this study with Tris extended Jersey semen was found to be lower than the observations of Mohanty *et al.* (1983) and Bhosrekar *et al.* (1992) which might be due to variation in season and fertility rate of bulls and females. The Biociphos plus diluent was able to produce a non significantly higher fertility rates over Tris diluent in both the breeds. However, Bohm *et al.* (1996) and Hinsch *et al.* (1997) reported higher conception rates than in the present study based on non return rates with Biociphos plus dilutor in Jersey bulls.

It is concluded that Biociphos plus is equally effective to that of Tris in maintaining the keeping quality and post thaw semen characteristics. It proved to be a better diluent than Tris in controlling bacterial growth in frozen semen. Hence Biociphos plus dilutor may be used as an alternative to Tris for the cryopreservation of bovine semen.

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