

Comparative performance of biociphos and egg yolk based extenders for buffalo semen cryopreservation*

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ABSTRACT

A study was conducted on 36 ejaculates from 6 Murrah buffalo bulls to compare the performance of biociphos extender with traditional egg yolk based extender. Semen samples were split into 2 parts and performance was evaluated at refrigerated temperature and -196°C . At refrigerated temperature no significant difference was observed for sperm motility, non eosinophilic counts, sperm abnormalities, intact acrosome status and HOST up to 48 h of preservation. However, total plate counts were significantly lower in biociphos than TYE at all durations of preservation. Similarly, no significant differences were observed in all seminal characteristics in pre-freeze and post-thaw semen samples diluted in biociphos and TYE. However, total plate counts (bacterial load) were significantly lower in biociphos as compared to TYE in both pre-freeze and post-thaw stage. Overall the study revealed that biociphos can be used to replace traditional TYE for buffalo semen preservation considering the better visibility in motility assessment and low bacterial load in biociphos as compared to TYE.

Key words: Biociphos, Buffalo semen, Cryopreservation, Semen, Soybean based extender

Production of quality semen, which is free from pathogens and having higher fertility is the major challenge faced by the AI industry. Egg yolk and milk are conventionally used in bovine semen cryopreservation owing to their protective effect against cold shock. However, both of these are products of animal origin thus their use introduces the risks of microbial contamination with the subsequent production of endotoxins capable of damaging the fertilizing ability of spermatozoa (Bousseau *et al.* 1998). Thun *et al.* (2002) and Stradaoli *et al.* (2007) investigated to replace egg yolk and milk in semen extenders and noticed that these can be effectively replaced with extenders containing soy lecithin. Therefore, present study was undertaken to evaluate the performance of soy lecithin based extender (biociphos) for buffalo semen preservability.

MATERIALS AND METHODS

The comparative performance of Tris Egg Yolk extender (TYE) and plant based extender (PBE), i.e. biociphos, was evaluated in terms of microbial load and seminal

characteristics. In the present investigation 36 ejaculates (initial motility $>70\%$) of 6 Murrah buffalo bulls maintained at Artificial Breeding Complex of the institute were used. Semen was collected twice a week from each bull using sterilized artificial vagina (inside temperature 43°C). Each ejaculate was split into 2 parts and processed in Tris egg yolk dilutor and biociphos dilutor for preservation at $+5^{\circ}\text{C}$ for 0, 24 and 48 h and other part was subjected to freezing (-196°C) for further analysis. Semen was analysed for motility, non-eosinophilic counts (Bloom 1950), sperm abnormalities, acrosome integrity (Hancock 1952) and plasma membrane integrity by hypo-osmotic swelling test (Jeyendran *et al.* 1984). The microbial load and sperm quality was evaluated in refrigerated (0, 24 and 48 h), and frozen (at -196°C) thawed semen. The bacterial count /ml of samples were estimated by multiplying the dilution factor with the mean number of colonies in media plates.

Bacterial load /ml = Bacterial colonies counted on plate
 $\times$ dilution factor

Data were analyzed by 2-way ANOVA with interaction and least squares technique (Harvey 1975). The percent data on semen characteristics were subjected to arcsine transformation and microbial count was subjected to log transformation for analysis (Snedecor and Cochran 1994).

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RESULTS AND DISCUSSION

Quality was assessed for semen preserved both at refrigerator temperature (5°C) and at -196°C and the results are presented in Tables 1 and 2.

Preservation of semen at 5°C

Least squares means of individual sperm motility, percent non-eosinophilic counts, HOS reacted spermatozoa and total plate counts in different treatment groups at 0, 24 and 48 h of preservation at refrigerated temperature are presented in Table 1. Though, the percent values of sperm motility after 0, 24 and 48 h of preservation at 5°C were higher in TYE than PBE, however the differences were not statistically significant at all durations of preservation. Analysis of variance revealed highly significant effect ($P<0.01$) of duration and extender. It was interesting to note that, subjectively, sperm motility and visualization of sperm were clearly improved when diluted with biociphos.

The result revealed that the average non-eosinophilic sperm (%) was significantly ($P<0.05$) higher in TYE than PBE at all durations of storage. Analysis of variance showed highly significant effect ($P<0.01$) of duration and extender.

No significant difference in sperm abnormalities was observed between extenders at different durations of preservation. Analysis of variance showed highly significant effect ($P<0.01$) of duration and extenders. Similar trends were observed for the head, mid-piece and tail abnormalities. Results of the study were in agreement to the Tuncer *et al.* (2005) who compared Laiciphose and AndroMed (soy based extender) extenders for maintaining sperm motility at 4°C in Holstein bulls. It was concluded that bull sperm diluted with both extenders can be used up to 3 days at +4°C for artificial insemination and no significant differences in sperm motility and viability were observed between extenders.

There was no significant difference observed in intact acrosome values between 2 extenders at different durations

of preservation. Overall analysis of variance showed highly significant ($P<0.01$) effect of duration and extender on intact acrosome values. The average HOST (%) estimates in semen in TYE were higher than those of PBE at all durations of preservation. However, the differences were not statistically significant. Analysis of variance showed highly significant effect ($P<0.01$) of duration and extenders on HOST.

The average microbial load ($10\times$) in semen was significantly ($P>0.01$) lower in PBE as compared to TYE at all durations of preservation. Analysis of variance showed highly significant effect ($P<0.01$) of duration and extenders on microbial load. Results related to microbial load were in agreement to those of Bousseau *et al.* (1998) who reported the lowest contamination in semen when diluted in soy lecithin based diluent (biociphos).

Preservation of semen at -196°C

Least squares means of individual sperm motility, percent non-eosinophilic counts, HOST percent and total plate counts in pre-freeze and post-thawed semen samples in different treatment groups are presented in Table 2. The percent individual motility values in pre-freeze and post-thaw semen were 78.39 and 55.84 for TYE; and 76.19 and 54.25 for biociphos, respectively. Pre- and post-freeze motility was higher in TYE as compared to PBE; however, the difference was not significant. Overall analysis of variance showed significant effect ($P<0.01$) of stage and extender on sperm motility. There was better clarity in motility assessment of ejaculates diluted in PBE than TYE. This may be attributed to less viscosity and hence more clarity of the PBE. However, in TYE, fat globules may interfere with the sperms.

In pre-freeze and post-thaw semen non eosinophilic sperm count was higher in semen diluted in TYE as compared to that of PBE; however, the differences were not significant. Overall analysis of variance revealed significant effect ($P<0.01$) of extender and stage.

Table 1. Semen characteristics (%) and bacterial load in refrigerated semen (up to 48 h) of Murrah bulls (N=36) preserved in tris and biociphos extenders

Semen characteristics	0 h				24 h				48 h			
	Tris		Biociphos		Tris		Biociphos		Tris		Biociphos	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
IM	78.39	0.00	76.19	0.01	62.55	0.00	60.55	0.00	50.53	0.01	45.36	0.01
N-eosin	87.15 ^a	0.00	84.30 ^b	0.00	77.68 ^a	0.01	73.62 ^b	0.01	64.91 ^a	0.01	53.36 ^b	0.01
<i>Sperm abnormalities</i>												
Head	1.72	0.00	2.06	0.00	1.97	0.00	2.66	0.00	2.84	0.00	3.60	0.00
MP	1.43	0.00	1.72	0.00	1.62	0.00	2.10	0.00	2.15	0.00	3.02	0.00
Tail	3.30	0.00	3.94	0.00	4.86	0.00	5.22	0.00	6.32	0.00	6.52	0.01
Total	6.53	0.01	7.79	0.01	8.54	0.01	10.09	0.01	11.42	0.01	13.26	0.01
Host	65.49	0.01	64.86	0.01	55.02	0.01	53.96	0.00	39.01	0.01	35.96	0.01
IA	90.98	0.01	90.51	0.01	77.97	0.01	76.34	0.00	61.02	0.01	56.38	0.01
TPC ($\times 10$)	210.37 ^a	1.11	106.13 ^b	1.10	182.23 ^a	1.11	46.24 ^b	1.10	162.98 ^a	1.11	20.85 ^b	1.09

Table 2. Semen characteristics (%) and bacterial load in pre-and post-freeze semen of Murrah bulls (N=36) preserved in Tris and biociphos extenders

Semen characteristics		Pre freeze semen				Post thaw semen			
		Tris		Biociphos		Tris		Biociphos	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
IM		78.39	0.00	76.19	0.01	55.84	0.01	54.25	0.01
N-eosin		87.15	0.00	84.30	0.00	65.01	0.01	63.59	0.01
Sperm Abnormalities	Head	1.72	0.00	2.06	0.00	2.38	0.01	2.57	0.01
	MP	1.43	0.00	1.72	0.00	1.96	0.01	2.40	0.01
	Tail	3.30	0.00	3.94	0.00	4.99	0.01	5.83	0.01
	Total	6.53	0.01	7.79	0.01	9.49	0.02	11.03	0.02
HOST		65.49	0.01	64.86	0.01	43.45	0.01	41.38	0.01
IA		90.98	0.01	90.51	0.01	65.88	0.01	65.78	0.01
TPC (x10)		210.37 ^a	1.11	106.13 ^b	1.10	121.64 ^a	1.11	18.89 ^b	1.09

IM: Individual sperm motility (%); N-eosin: Non-eosinophilic count (%); MP: mid piece; HOST: hypo-osmotic swelling reactivity (%); IA: intact acrosome (%); TPC: total plate count (X10) (cfu/ ml). Values with different superscripts within a row differ significantly.

No significant difference in sperm abnormalities was observed between extenders at different stages. Overall analysis of variance revealed highly significant ($P<0.01$) effect of stage and significant effect ($P<0.05$) of extender on sperm abnormalities. Similar trends were observed for the head, mid-piece and tail abnormalities. Overall the result showed that the spermatozoal abnormalities were increased after cryopreservation. The fewer abnormalities were found in TYE than PBE at all stages of semen preservation. Under this study, the tail abnormalities increased after freezing as compared to fresh semen sample. Higher tail abnormality in the frozen semen may be due to the freezing stress during cryopreservation.

Percent intact acrosome values were higher in semen diluted in TYE but the differences were not significant. Analysis of variance revealed highly significant effect ($P<0.01$) of stage of preservation on intact acrosome values. Results of the present study were in accordance with those of Hinsch *et al.* (1997) and Gil *et al.* (2000) who observed no significant differences in sperm viability, acrosomal status and sperm motility of semen samples in either biociphos or commercial egg yolk based extender.

No significant difference in HOST percent values was observed between extenders in pre-freeze and post-thaw semen. Analysis of variance revealed highly significant ($P<0.01$) effect of stage of preservation on sperm HOST values. Results of the present study indicated that biociphos, when used as an extender for freezing of buffalo semen, was not inferior in preservation of plasma membrane integrity to TYE. On the similar lines Thun *et al.* (2002) examined egg yolk based extenders vis-à-vis biociphos plus and reported that Tris extenders containing egg yolk resulted in superior cryoprotective effects as compared to soybean based extender biociphos however, the differences in sperm characteristics were not significant between different extenders.

The microbial load values ($\times 10$) in pre-freeze and post-thaw semen were 210.37 and 106.13 in TYE; and 121.64 and 18.89 in PBE, respectively. In both pre-freeze and post-thaw semen PBE revealed significantly lower ($P<0.01$) microbial load as compared to TYE. Analysis of variance revealed highly significant effect ($P<0.01$) of extender and stage of preservation. Our results were in agreement to those of Bousseau *et al.* (1998) who reported that the 2 diluents (triladyl and laiciphos) containing animal products showed moderate (10 to 60 CFU/ml) contamination with bacteria or mycoplasma while no contamination was observed in lecithin containing diluent (biociphos).

An overview of present study suggested that the use of biociphos resulted in significantly less microbial load as compared to that of TYE and no significant differences in motility, viability, maintaining acrosome and plasma membrane status when compared to traditional egg yolk based extender. Our results indicated that biociphos may be successfully used for buffalo semen preservation both at refrigerated temperature and in liquid nitrogen considering better visualization of sperm and low bacterial load when diluted with biociphos.

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