

## Effect of prostaglandin $F_2\alpha$ , oxytocin and butylated hydroxytoluene on enzyme activities of crossbred (HF x Haryana) bull semen

A. ARANGASAMY<sup>1</sup>, L.P. SINGH<sup>2†</sup>, M.R. ANSARI<sup>3</sup>, O.P. GUPTA<sup>4</sup> AND G.S. BISHT<sup>5</sup>

Animal Reproduction Division  
Indian Veterinary Research Institute  
Izatnagar-243 122 (UP) INDIA

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### ABSTRACT

Semen samples were collected from two crossbred bulls (HF x Haryana) and diluted in tris with three combinations of additives i.e., prostaglandin  $F_2\alpha$ , oxytocin and butylated hydroxytoluene (2mM/ml) and frozen in liquid nitrogen. The enzyme activities (GOT, GPT, ACP and AKP) were evaluated at prefreeze and postfreeze stage. Except GPT the other enzymes showed no significant difference between treated and control groups at prefreeze stage. The value of GOT and GPT differed significantly between treatment and control groups, whereas, there was no significant difference in ACP and AKP at postfreeze stage. The enzymatic activity in the post-thaw semen treated with these additives revealed a lower leakage of enzyme than control.

**Key Words:** Bull, semen, transaminases, phosphatase,  $PGF_2\alpha$ , oxytocin, BHT

Glutamic oxaloacetic transaminase (GOT) and Glutamic pyruvic transaminase (GPT) are important transaminases present in semen which are concerned with oxidative metabolism. The GOT activity is mainly associated with the sperm cell as well as seminal plasma and its amount in seminal plasma arose chiefly from leakage of spermatozoa (Pace and Graham, 1970). The phosphatase, an enzyme in semen is an indicative of functional state of accessory sex gland and metabolic activity of sperm (Abdou *et al.*, 1974). The estimation of enzyme activities in seminal plasma during prefreeze and postfreeze condition reflect sperm membrane integrity. The present study was conducted to assess transaminases and phosphatases activities of bull semen treated with prostaglandin  $F_2\alpha$ , oxytocin and butylated hydroxytoluene (BHT).

Twenty four ejaculates (12 each from two bulls) from crossbred bulls (HF x Haryana) maintained under identical feeding and managerial regimen were used for the study. Semen was

collected in A.V. twice a week. The ejaculates were split into 4 parts. Each part was diluted separately with Tris+ additive combinations @ 1.5 ng/ml  $PGF_2\alpha$ , 0.1 units/ml oxytocin) as per the method described by Lokanathan, 1993 and BHT 2mM/ml as per Killian *et al.* (1989) respectively, and the aliquot was kept at control. Egg yolk and glycerol were added @ 20 and 7%, respectively. The four split samples were frozen in medium french straw after providing combined cooling cum equilibrium period of 4 hrs. Seminal plasma was separated by centrifugation at 3000 rpm for 20 min. The plasma was separated out and preserved in sterilized plastic vials at  $-20^\circ\text{C}$  until assayed for enzyme activity. Transaminases and phosphatases were estimated as per the method of King and Armstrong (1934), respectively. Statistical analysis of the data was done according to the method described by Snedecor and Cochran (1989).

The result of present study (Table) revealed that at prefreeze stage except GPT, other enzymes (GOT, ACP and AKP) showed no significant difference between treatment and control groups. At postfreeze stage GOT and GPT differed significantly ( $P < 0.05$ ) between treatment and control groups.

<sup>1</sup>Ph.D. Scholar, <sup>2</sup>Senior Scientist, <sup>3</sup>Principal Scientist, <sup>4</sup>Principal Scientist, Veterinary Surgery, <sup>5</sup>Principal Scientist, Statistics

<sup>†</sup>Corresponding author

**Table 1:** Mean (±SE) prefreeze and post-freeze, GOT, GPT, ACP and AKP levels of bull seminal plasma frozen with various semen additives

Enzymes	Stage of processing	Bull No.	Control	Prostaglandin F <sub>2</sub> α	Oxytocin	BHT
GOT (μmole/L)	Pre-freeze	Overall	21.90±0.75	19.41±0.75	19.63±0.72	20.39±0.78
	Post-freeze	Overall	30.95±0.63 <sup>a</sup>	25.063 <sup>b</sup>	25.55±0.53 <sup>b</sup>	25.22±0.42 <sup>b</sup>
GPT (μmole/L)	Pre-freeze	Overall	5.45±0.11 <sup>a</sup>	4.53±0.13 <sup>b</sup>	4.88±0.18 <sup>b</sup>	4.69±0.16 <sup>b</sup>
	Post-freeze	Overall	10.53±0.25 <sup>a</sup>	9.13±0.24 <sup>b</sup>	9.75±0.29 <sup>b</sup>	9.26±0.26 <sup>b</sup>
ACP (KAU/100 ml)	Pre-freeze	Overall	424.95±49.83	401.30±50.73	409.42±42.28	400.63±50.50
	Post-freeze	Overall	524.61±54.66	499.56±54.59	516.41±54.50	501.06±54.94
AKP (KAU/100 ml)	Pre-freeze	Overall	366.83±48.09	343.43±47.82	361.47±47.92	346.81±48.38
	Post-freeze	Overall	457.61±49.05	431.17±49.10	448.36±49.61	436.61±49.51

Mean bearing different superscript in the same column differ significantly ( $P < 0.05$ )

control groups. In control group, the values obtained in this study are in agreement with Belorkar *et al.* (1988), Lokanathan (1993) and Dhami and Sahni (1995). The damage to sperm cell membrane as revealed by the release of GOT and GPT during extension and freezing was minimum in semen added with oxytocin, PGF<sub>2</sub>α than control indicates the protective action of PGF<sub>2</sub>α in sperm membrane. The similar findings has been obtained by Lokanathan (1993) and Daadar *et al.* (1987). Oxytocin treated groups also reflected the similar results as it was observed in PG group. There was significantly ( $P < 0.05$ ), lesser release of GOT and GPT in BHT treated samples than control. This might be due to binding of BHT with sperm cell membrane and minimizing the enzyme release. Phosphatases (ACP and AKP) activity in the present study revealed a non-significant difference between treatment and control groups at prefreeze and postfreeze stages. It can be concluded that the addition of PGF<sub>2</sub>α, Oxytocin and BHT to semen prior to freezing minimize the transaminases (GOT and GPT) leakage than phosphatases (ACP and AKP) significantly ( $P < 0.05$ ), thus reducing the sperm cell damage and increased viability.

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