HEPARIN BINDING PROTEIN IN SEMEN OF BUFFALO BULL MIGHT BE RESPONSIBLE FOR BETTER SEMEN FREEZABILITY AND SUBSEQUENT FERTILITY

A.K. SINGH[1]*, P.S. BRAR[2] AND R.S. CHEEMA[3]

Department of Veterinary Gynaecology and Obstetrics
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana - 141 004

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ABSTRACT

The present study determined the concentration of purified heparin binding proteins (HBP) in frozen-thawed spermatozoa of buffalo bulls and established the relationship of HBP with semen characteristics and fertility. About 50 frozen semen straws of same ejaculate per buffalo bull (n=30 bulls) were used to establish purified HBP concentrations in frozen-thawed spermatozoa extracts. On the basis of HBP, all the bulls were grouped as high fertility (HFB, ≥0.3 mg/10^9 sperms; n=15) or low fertility (LFB, <0.3 mg/10^9 sperms; n=15) bulls. The frozen-thawed semen was evaluated for % acrosome reaction, HOST, spermatozoa viability and motility, as well as first service conception rate (FSCR). The results revealed that purified HBP concentration and semen characteristics like % acrosome reaction, motility and FSCR were higher ($p<0.05$) in HFB than in LFB. In conclusion, the higher concentrations of HBP in buffalo bulls might be responsible for their better semen quality and fertility.

Keywords: Acrosome reaction, Buffalo bull, FSCR, Heparin binding proteins, Frozen semen

INTRODUCTION

The selection of high fertility bulls is a challenge since large variations exists in the result of tests applied for semen evaluation. Till date, there is no single objective test that could precisely evaluate the fertility potential of spermatozoa. The search is now on for finding the molecular markers of semen fertility (Singh *et al.,* 2014). Several factors in seminal plasma and/or spermatozoa have been investigated like heparin binding proteins (HBP), heat shock protein, clusterin, spermadhesin, osteopontin and many other unidentified proteins that modulate the fertilizing ability of spermatozoa (Asquith *et al.,* 2005). Among these, HBP and their close associates represent a superfamily of proteins in bovine. Five major HBP proteins with 18 - 55 kDa molecular weight (18, 20, 24, 31, 55 kDa) were identified in bovine seminal plasma that participate in sperm function and were named as fertility associated antigen-5-complex (McCauley *et al.,* 2001).

Previous studies have shown that an increase in spermatozoa motility and viability, acrosomal integrity and hypo-osmotic swelling test (HOST) were associated with an increase in HBP-treated group (Harshan *et al.,* 2006). It was further suggested that for optimum fertility, HBP levels on the surface of spermatozoa in a semen sample should be 0.3 mg/10^9 sperms (Chacur *et al.,* 2010). Moreover, the determination of HBP in cryopreserved sperm extracts gives a more legitimate picture, owing to actual protein availability for post-thaw acrosome reaction and penetrations of oocyte vestments (Nauc and Manjunath, 2000). However, the concentrations of HBP in relation to frozen semen quality and fertility are still not fully understood in buffalo bulls. Therefore, keeping in view of the above cited facts and the deficit knowledge of HBP concentrations in buffalo bulls, the present study was undertaken to determine the concentrations of purified HBP in frozen-thawed semen *vis-à-vis* semen characteristics and fertility of breeding buffalo bulls.

[1]Assistant Professor, [3]Senior Physiologist (Reproduction); [2]Dean, College of Veterinary Science; *assengar2001@yahoo.co.in

MATERIAL AND METHODS

Thirty healthy Murrah breeding buffalo bulls maintained under identical conditions of feeding and management at two government semen processing and freezing laboratories were used for collection of semen (September: ambient temperature 30.6^{0}C, relative humidity 92%). Semen (50 straws per bull) frozen from same ejaculate was procured from all bulls and earmarked for further investigations. The frozen-thawed semen (20 straws per bull) was centrifuged at 3000 rpm for 10 minutes to separate out dilutor. The sodium dodecyl sulphate-sperm extracts (SDS-SE) from remnant sperm pellet of frozen-thawed semen were stored in 0.5 ml fractions at -20^{o}C till further use.

In frozen-thawed semen, the HBP were purified using heparin-sepharose affinity chromatography (Kraus *et al.,* 2001). The area under HBP curve was calculated by Simpson's 1/3rd rule (Jain *et al.,* 1993). On the basis of purified HBP concentration obtained from frozen-thawed sperm extracts, all bulls were divided as high fertility bulls (HFB, ≥0.3 mg/10^9 sperms; n=15) and low fertility bulls (LFB, < 0.3 mg/10^9 sperms; n=15).

The frozen-thawed semen from same ejaculate was evaluated for percent acrosome reaction, HOST, viability and motility (total and progressive) using computer assisted semen analysis (CASA; version Hamilton-Thorne IVOS 12.2). Five randomly selected fields were scanned per straw and five straws per bull semen were evaluated to denote motility, obtaining 25 scans for each bull. The mean of 25 scans for motility (total and progressive) and the mean of three replicates for percent acrosome reaction, HOST and viability per bull semen were used for the statistical analysis.

Three hundred buffalo were enrolled for fixed time artificial insemination (FTAI) program ($PGF_{2\alpha}$-GnRH-$PGF_{2\alpha}$-GnRH on day -2, 0, 7 and 9, respectively followed by AI at 16 and 40 h after last GnRH injection) using frozen semen to determine the fertility of bulls. The percent first service conception rate (FSCR) was calculated by dividing the number of buffalo conceived after first insemination with total number of first services.

The statistical analysis was performed with Statistical Package for Social Sciences (SPSS, version 16.0) program. The proportionality data were transformed using the arcsine transformation with adjustment to allow for zero values. The mean±SE were calculated using arcsine-transformed data in the software. Duncan's multiple range test and one way analysis of variance (ANOVA) was used for comparing the level of significance among the group of bulls of different gradients (HFB and LFB). The minimum significant interaction was considered at 5% level.

RESULTS AND DISCUSSION

The purified HBP concentrations in frozen-thawed sperm extracts were higher ($p<0.05$) in HFB than in LFB (Table 1; Fig. 1). Average HBP concentrations seen in this study were similar to an earlier report in buffalo bulls (Arangasamy *et al.,* 2005; 0.361 mg/10^9 sperms), but are much lower than those in cattle semen (Srivastava *et al.,* 2012; 27.9 mg/ml). This difference in HBP levels could merely be a species variation and/ or inherent character. Previous studies (McCauley *et al.,* 2001) have shown that HBP are primarily produced from accessory sex glands, secreted into seminal fluid and bound to sperm at ejaculation. Similarly, the area under HBP curve was greater ($p<0.05$) in HFB (144.3 ± 4.4 mm^2) than in LFB (126.5 ± 3.9 mm^2). Limited studies in buffalo bulls have revealed that HBP concentrations were drastically suppressed in bulls with sub-fertility (Singh *et al.,* 2014).

In frozen-thawed semen, motility (total and progressive), acrosome reaction and FSCR were higher ($p<0.05$) in HFB as compared to LFB semen sample (Table 1). The reduction in post-thaw motility in LFB semen might possibly be due to changes in membrane fluidity because of lower HBP, which makes sperm plasma membrane more prone to damage during freezing and thawing process (Kumar *et al.,* 2008). A

Table 1: Heparin Binding Protein (HBP) concentrations, sperm characteristics and fertility parameters in semen of high fertility (HFB) and low fertility (LFB) buffalo bulls

Parameters	HFB (n=15)	LFB (n=15)
HBP-FTSE, mg/10^9 sperms	0.46±0.02[a]	0.25±0.01[b]
Area under HBP-FTSE curve, mm^2	144.3±4.4[a]	126.5±3.9[b]
Total motility, %	59.3±2.2[a]	51.7±2.0[b]
Progressive motility, %	31.8±1.6[a]	27.3±1.7[b]
Acrosome reaction, %	54.9±2.8[a]	49.2±2.3[b]
HOST, %	68.5±1.7	67.2±2.1
Viability, %	67.5±2.2	71.2±2.2
FSCR, %	42.7±4.3[a]	31.3±4.3[b]

[a *vs.* b]p<0.05, within a row; HOST, Hypoosmotic swelling test; FSCR, First service conception rate; HBP-FTSE, Heparin binding proteins in frozen-thawed sperm extract

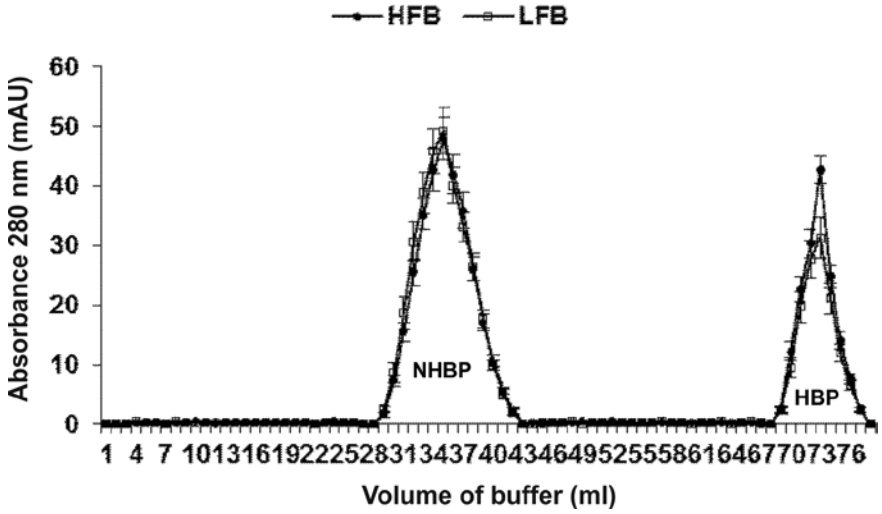

Figure 1: Heparin Binding Protein (HBP) and Non-HBP concentrations in frozen-thawed sperm extracts of high and low fertility buffalo bulls (HFB, LFB) separated by heparin-affinity chromatography

higher percent acrosomal reaction in HFB is similar to an earlier report by Rasul *et al.* (2001). At ejaculation, HBP binds to sperm membrane capacitation factors viz. heparin and glycosaminoglycans, resulting in capacitation, acrosome reaction, sperm oocyte fusion and fertilization (Divyaswetha *et al.,* 2008). Eventually, the higher levels of HBP in high fertility bulls might also contribute toward higher capacitation status in the present study. Therefore, HBP play a vital role in capacitation process and were indicated as a biochemical marker to predict the fertility potential of bulls. Likewise, FSCR was also higher (p<0.05) higher in HFB than in their counterparts. Similar studies in cattle bulls have recorded 15-17% higher pregnancy rates in females inseminated with HBP-positive spermatozoa than those inseminated with HBP-negative spermatozoa (Sprott *et al.,* 2000). It was postulated that poor reproductive performance in bulls could be partly due to lower levels of HBP (Harshan *et al.,* 2006). Alternatively, the absence of difference (p<0.05) was observed in the percentage of viable sperms and HOS-reactive spermatozoa in present

study, which was in agreement with previous studies (Ansari *et al.,* 2011).

Thus, high concentrations of HBP in semen of bulls with high fertility might be responsible for its better freezability and fertility as reflected by increased post-thaw motility, acrosome reaction and FSCR.

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