Ciência Rural, Santa Maria, v.39, n.1, p.275-278, jan-fev, 2009
ISSN 0103-8478

Heparin-binding proteins of seminal plasma in Nellore bulls

Proteínas ligadoras à heparina do plasma seminal em touros Nelore

Carlos Eurico Fernandes[I] Fabiana Ferreira de Souza[II] Jayme Augusto Souza-Neto[III] Paulo Eduardo Martins Ribola[IV]

- NOTE -

ABSTRACT

*The aim of this study was to identify heparin-binding proteins (HBPs) in seminal plasma of Nellore (**Bos taurus indicus**) bulls. Bulls (n=4), 30-36 months old, 500-550kg with satisfactory seminal quality were selected. After the centrifugation, samples of the seminal plasma were pooled and the HBPs were isolated by heparin-affinity chromatography. The recovered HBPs fractions were pooled. One-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDSPAGE) 12.5% was performed in vertical minigels. Eight bands with molecular weights ranging from 15 to 63kDa were observed. Two proteins were identified (22 and 25kDa), similar to those previously described in **Bos taurus taurus** bulls. Other bands identified in this study (39, 53, 58 and 63kDa) have not been previously observed and possibly they are specific to Nellore semen.*

Key words: *bovine, proteins, seminal plasma, **Bos taurus indicus**.*

RESUMO

*O objetivo deste estudo foi identificar proteínas ligadoras à heparina no plasma seminal de touros Nelore (Bos taurus indicus). Para tanto, foram selecionados quatro touros entre 30 e 36 meses de idade e peso aproximado de 500-550kg. Após centrifugação, amostras do plasma seminal foram misturadas e as proteínas ligadoras à heparina foram isoladas por meio da cromatografia por afinidade. As frações após a eluição foram agrupadas para caracterização das bandas protéicas (SDSPAGE, 12,5%). Foram identificadas oito bandas protéicas variando entre 15 e 63kDa. Duas proteínas com 22 e 25kDa foram similares às descritas em touros **Bos taurus taurus**. Outras proteínas identificadas com 39, 53, 58 e 63kDa ainda não foram descritas e possivelmente sejam específicas para **Bos taurus indicus**.*

Palavras-chave: *bovinos, proteínas, plasma seminal, **Bos taurus indicus**.*

In the last two decades there has been important progress in the identification and characterization of many components of seminal glands secretions, resulting in new perspectives regarding to the analysis of domestic animal ejaculated. Perhaps the most significant discovery has been the participation of heparin-binding proteins (HBPs) in the sperm capacitation (THÉRIEN et al., 1995). The spermatozoa are able to bind to heparin and also similar molecules in the oviduct. This ability is attributed to the presence of seminal plasma proteins, which are attached to the sperm surface after ejaculation, leading to modulation of the acrosomal reaction by zona pellucida (ZP) glycoproteins (THÉRIEN et al., 1997).

Nellore (***Bos taurus indicus*)** cattle represent most of the Brazilian beef herd, around 160 million

[I]Departamento de Patologia, Centro de Ciências Biológicas e da Saúde (CCBS), Universidade Federal de Mato Grosso do Sul (UFMS), CP 549, 79070-900, Campo Grande, MS, Brasil. E-mail: cefernandes@nin.ufms.br. Autor para correspondência.
[II]Hospital Veterinário, Universidade de Franca (UNIFRAN), Franca, SP, Brasil.
[III]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, USA.
[IV]Departamento de Parasitologia, Instituto Biológico, Universidade Estadual de São Paulo (UNESP), Campus de Botucatu, Botucatu, SP, Brasil.

Received 05.06.08 Approved 07.23.08

animals. However, few studies have considered aspects of sperm physiology or seminal electrophoretic profile. Although all HBPs may bind to sperm surfaces, only one has been correlated with greater fertility potential (BELLIN et al., 1996, 1998). Thus, HBPs have been indicated as a biochemical marker to predict the fertility potential of bulls. The object of this study was to separate the HBPs from Nellore seminal plasma using heparin-affinity chromatography.

In this study, bulls (n=4), 30-36 months old, 500-550kg were selected by seminal quality (>80% of gross motility and >90% normal sperm morphology) and maintained in semi-confinement. The animals were submitted to daily semen collection by electroejaculation for three days and after rest of five days, a simple ejaculate was obtained. Seminal plasma was separated from spermatozoa by centrifugation at 4.200 x g for 1h at 4°C. After, 200µL samples of the seminal plasma were pooled. The heparin-binding proteins were isolated by heparin-affinity chromatography[a] as described by MANÁSKOVÁ et al. (2002) and modified as follows: the pooled seminal plasma (800µL) was added into a heparin-affinity chromatography column (16mm x 26mm, 1.0mL min^{-1}) and equilibrated with PBS. Non-adsorbed proteins were washed out with PBS and bound proteins were eluded with 1M NaCl. Analysis of separated proteins was calculated using specialized software[b]. The recovered heparin-binding proteins fractions were pooled in agreement with the observed curve (Figure 1). One-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDSPAGE, 12.5%) was performed in vertical minigels[c] (de SOUZA, 2003). The gels were stained with coomassie blue, scanned and molecular weight (kDa) values for each band within a lane were calculated using image analysis software[d]. The electrophoresis analysis of the pooled eluted fractions identified 8 bands, with molecular weights ranging from 250 to 15kDa (Figure 2).

Previous studies using one-dimensional polyacrylamide gel electrophoresis have detected between 25 and 32 bands in the seminal plasma of Nellore bulls (FERNANDES et al., 2005) and about 30% of seminal plasma bands bind to heparin and are associated with the sperm capacitation process (MILLER et al., 1990). Five families of HBPs with different affinity for heparin constitute complexes with molecular weight ranging from 14 to 31kDa in ***Bos taurus taurus*** bulls (MILLER et al., 1990; BELLIN et al.,

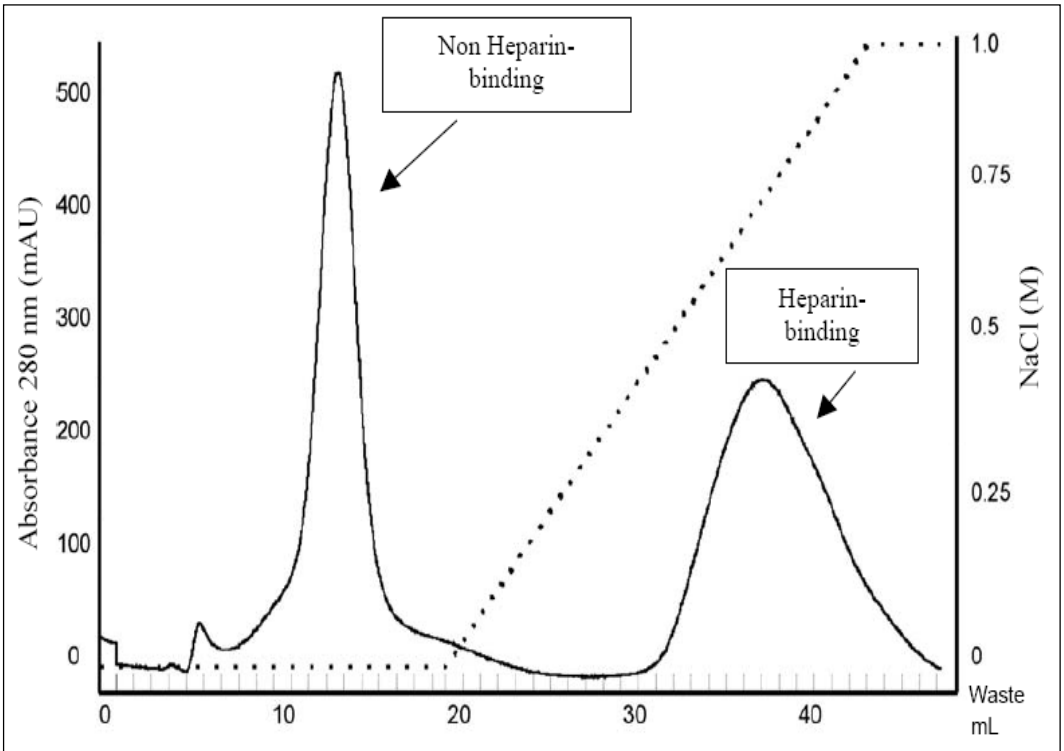

Figure 1 - Graphic image plotted by UNICORN Control System software (***Amersham Biosciences***, Uppsala, Sweden) of heparin-binding and non-heparin-binding proteins in seminal plasma of Nellore bulls separated on heparin-affinity chromatography.

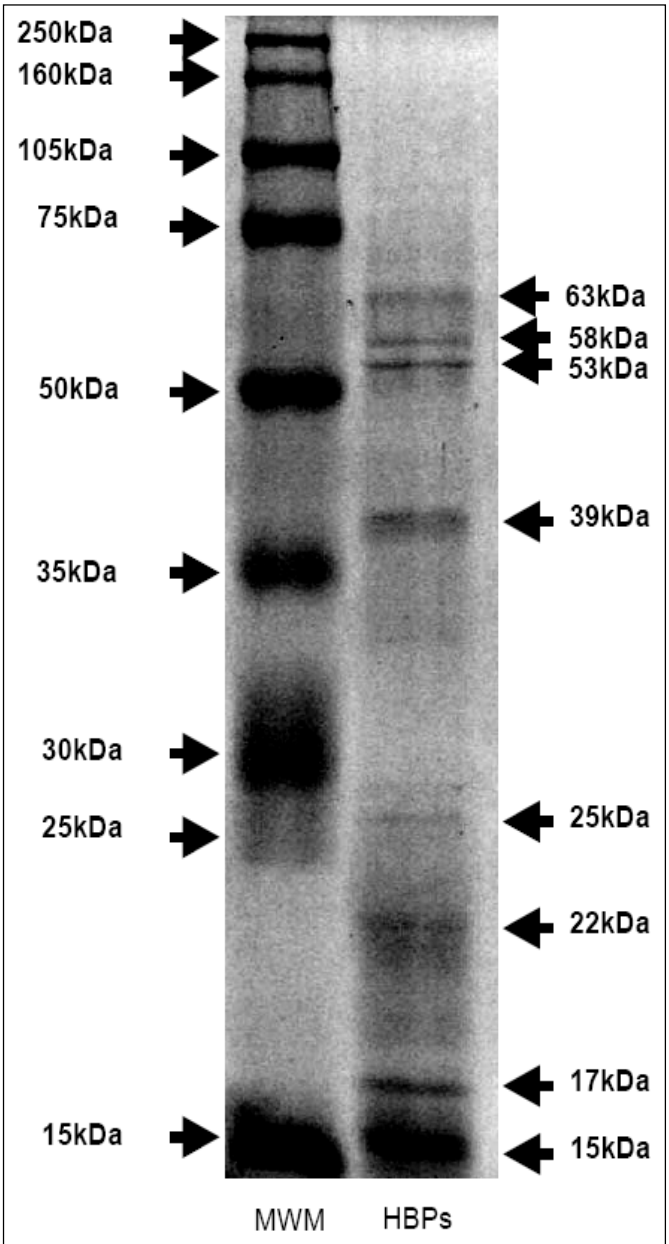

Figure 2 - Polyacrylamide electrophoresis gel (SDS-PAGE) at 12,5% in a discontinuous alkaline system of eluted fractions of heparin-binding proteins (HBPs) of seminal plasma of Nellore bulls. MWM holds for molecular weight marker.

1996). In the present study, eight bands from 15 to 63kDa bound to heparin. Recently, ARANGASAMY et al. (2005) also observed eight HBPs in fresh semen of buffalos (***Bubalus bubalis***). This preliminary observation is supported by the detection of multiple spermatozoal proteins, more specifically between 21.5 to 24kDa, in association with acrossome reaction (BELLIN et al., 1996; MCCAULEY et al., 1996). The current identified two proteins in Nellore seminal plasma with 22 and 25kDa, probably with analogous proprieties. However, their relationship with fertility remains to be determined. The 25kDa proteins are possibly correlated with tissue inhibitor of metalloproteinases-2, previously purified from seminal fluid (McCAULEY et al., 2001). However, the mechanism involved in regulation of bull sperm physiology is unknown. Additionally, 15 and 17kDa HBPs had high concentration, as demonstrated by densitometry, and could be the same as those previously observed by MILLER et al. (1990). These proteins have an affinity for the plasma membranes of sperm after ejaculation and are secreted from seminal vesicles. Other bands founds in this study (39, 53, 58 and 63kDa) were not previously described and perhaps they are specifics to semen of ***Bos taurus indicus*** bulls. The ability to bind and respond to glycosaminoglycans (GAGs) in the oviduct is related to semen quality and fertility. Thus, the characterization of seminal proteins and the study of their binding properties will be the first step in understanding their role in the fertilization process. The identification of HBPs would provide information that could improve our knowledge of this aspect of reproductive physiology in Nellore bulls.

SOUCERS OF MANUFACTURES

[a]Äkta™ Oligopilot, Amersham Biosciences
[b]UNICORNTM, Amersham Biosciences
[c]Mini VE®, Amersham Biosciences
[d]Image Master®, Amersham Biosciences.

REFERENCES

ARANGASAMY, A. et al. Isolation and characterization of heparin and gelatin binding buffalo seminal plasma proteins and their effect on cauda epididymal spermatozoa. **Animal Reproduction Science**, Champaing, v.90, p.243-254, 2005.

BELLIN, M.E. et al. Monoclonal antibody detection of heparin-binding proteins on sperm corresponds to increased fertility of bulls. **Journal of Animal Science**, Champaing, v.74, n.1, p.173-182, 1996.

BELLIN, M.E. et al. Fertility-associated antigen on bull sperm indicates fertility potential. **Journal of Animal Science**, Champaing, v.76, n.8, p.2032-2039, 1998.

De SOUZA, F.F. **Caracterização eletroforética do perfil protéico e análise bioquiímica do plasma seminal canino**. 2003. 98f. Tese (Doutorado em Medicina Veterinária) - Curso de Pós-graduação em Medicina Veterinária, Universidade Estadual Júlio de Mesquita Filho, Botucatu.

FERNANDES, C.E. **Perfil eletroforético, características estruturais e fertilidade in vitro do sêmen de touros Nelore submetidos à degeneração testicular por insulação**. 2005. 106f. Tese (Doutorado em Medicina Veterinária) - Curso de Pós-graduação em Medicina Veterinária, Universidade Estadual Júlio de Mesquita Filho, Botucatu.

MANÁSKOVÁ, P. et al. Isolation of non heparin-binding and heparin-binding proteins at boar prostate. **Journal of Chromatography B**, Amsterdam, v. 770, n.1, p.137-143, 2002.

McCAULEY, T.C. et al. Localization of a heparin-binding protein to distinct regions of bovine sperm. **Journal of Animal Science**, Champaing, v.74, p.429-438, 1996.

McCAULEY, T.C. et al. Identification of a heparin-binding protein in bovine seminal fluid as tissue inhibitor of metalloproteinases-2. **Molecular Reproduction Development**, v.58, p.336-341, 2001.

MILLER, D.J. et al. Heparin-binding proteins from seminal plasma bind to bovine spermatozoa and modulate capacitation by heparin. **Biology of Reproduction**, v.42, n.6, p.899-915, 1990.

THÉRIEN, I. et al. Phosphatidylcholine-binding proteins of bovine seminal plasma module capacitation of spermatozoa by heparin. **Biology of Reproduction**, v.52, n.6, p.1372-1379, 1995.

THÉRIEN, I. et al. Major proteins of bovine seminal plasma modulate sperm capacitation by high-density lipoprotein. **Biology of Reproduction**, v.57, n. 5, p.1080-1088, 1997.