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EXTENDED ABSTRACTS

Purification and Characterization of Heparin Binding Proteins from Seminal Plasma of Cross- bred Cattle Bulls by Affinity Chromatography, SDS-PAGE and Mass Spectrometry

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ABSTRACT

Heparin binding proteins (HBP) play an important role within the fertility of bovine semen. during this study HBPs purified from cross-bred cattle bull seminal plasma (SP) by sepharose-affinity chromatography were characterized by SDS-PAGE, and mass- spetrometery. Affinity chromatographic analysis indicated two peaks of unbound (non-HBP) and bound proteins (HBP). on the average , seminal plasma of cross-bred bulls contained 39.36 ± 4.41% HBP with a peak area of two .74 ± 0.82 cm2. Sixteen bands with molecular weights starting from 14 kDa to 150 kDa might be separated by SDS-PAGE from seminal plasma of 11 bulls. SDS-PAGE analysis of the eluted HBP peaks identified 14 bands, with molecular weights starting from 14 kDa to 150 kDa. However, variation in number of bands, separated in SP and SP-HBP was observed among the bulls. The matched peptides of 60; 40, 35; 31, 28; 25 and 20 kDa proteins with highest score (>61-67) were identified to possess significant matching with the peptides of Platelet activatin factor AH; Clusterin preprotein; DNase1-L3 and TIMP-2, respectively. This study envisaged that four characterized SP-BHPs have important functions in reproduction. Moreover, role of DNASE-1L-3 and TIMP-2 kDa proteins is said to higher conception rate of bovine. Therefore, this study opens an extra scope to research these SP – HBP as potential biomarkers of fertility in cross – bred bulls. Male reproductive efficiency relies on the power to mate with the feminine and fertilise the oocyte. Mating ability includes libido and physical capability to mount, achieve intromission and ejaculate (Parkinson 2004). so as to fertilise the oocyte, each spermatozoa must present motility, active mitochondria, intact membranes and a nucleus capable of proper decondensation and reorganisation. These attributes will allow spermatozoa to succeed in , recognise, bind to and penetrate the oocyte so as to deliver its genome. within the dairy industry, where AI (AI) is that the standard, male fertility is defined because the percentage of females inseminated that aren't re-inseminated an outlined number of days after the primary insemination. This fertility quantification is named non-return rate (NRR). during this context, NRR will only ask the sperm fertilising ability. Moreover, in AI with cryopreserved semen, ability of the spermatozoa to survive the cryopreservation process is additionally a part of the fertility definition (Collin et al. 2000). Each of the only steps resulting in fertilisation represents a juncture where defect could alter semen fertility. supported their implication especially steps of the fertilisation process, many sperm components like lipids, proteins, ions and nucleic acids are proposed to vary in quantity or quality consistent with the male fertility status in many mammalian species. Levels of P25b, a bovine sperm membrane antigen, are lower in semen from subfertile bulls than within the semen from bulls with high fertility (HF) rates. P25b counterparts in human and hamster, P34H and P26h, are involved in zone (ZP) recognition. A 30-kDa heparin-binding protein, namely fertility-associated antigen (FAA), characterised sperm membranes of beef bulls with greater fertility potential. FAA was further identified as DNase I-like protein. The bovine sperm proteome consists of or related to many different proteins. Although the functions of the bulk remain unknown, many of them must be involved especially steps of fertilisation. From now of view, absence, presence, under- or over-expression of specific proteins could alter sperm functions, jeopardising its fertilising abilities, thus lowering the semen fertility. the target of this study was to match the proteome of sperm Triton X-100 extracts from fertile and subfertile bulls and quantify differences by the two-dimensional difference gel electrophoresis (2D-DIGE) technique so as to spotlight putative subfertility explanations. Seminal plasma Binder of SPerm (BSP) proteins bind to sperm at ejaculation and promote capacitation. When in excess, however, BSP proteins damage the sperm membrane. it's been suggested that milk components of semen extenders accompany BSP proteins, potentially protecting sperm. Thus, this study was conducted to research if milk proteins interact with BSP proteins and reduce BSP binding to goat sperm. Using gel filtration chromatography, milk was incubated with goat seminal plasma proteins and loaded onto columns with and without calcium. Milk was also fractionated into parts containing mostly whey proteins or mostly caseins, incubated with seminal plasma proteins and subjected to gel filtration. Eluted fractions were evaluated by immunoblot using anti-goat BSP antibodies, confirming milk protein-BSP protein interactions. As determined by ELISA, milk proteins coated on polystyrene wells sure to increasing of goat BSP proteins. Far-western dot blots confirmed that BSP proteins sure to caseins and Î²-lactoglobulin during a concentration-dependent manner. Then, cauda epididymal sperm from five goats was incubated with seminal plasma; seminal plasma followed by milk; and milk followed by seminal plasma. Sperm membrane proteins were extracted and evaluated by immunoblotting. The pattern of BSP binding to sperm membrane proteins was reduced by 59.3Â that when epididymal sperm were incubated with seminal plasma then with skim milk (pâ€‰â€‰0.05). last , goat BSP proteins have an affinity for caseins and whey proteins. Milk reduces BSP binding to goat sperm, depending whether or not sperm had been previously exposed to seminal plasma. Such events may explain the protective effect of milk during goat sperm preservation.

Keywords: HBP; Characterization; Mass spectrometry; Seminal plasma; Bulls

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