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Heparin-binding proteins of canine seminal plasma

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Abstract

Heparin-binding proteins (HBP) from seminal plasma have been expected to participate in modulation of the acrosomal reaction, and have been correlated with fertility in some species. However, they have not been described in the dog. The aim of this study was to document the HBPs of canine seminal plasma. Six pooled samples of seminal plasma from three crossbred dogs were used. The HBPs were isolated by heparin affinity chromatography and the fractions recovered were pooled. One-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on 12 and 18% vertical minigels. The stained gels were scanned and the molecular weight (kDa) values for each band within a lane were calculated by image analysis software. The electrophoresis analysis of the pooled eluded fractions identified 19 bands, with molecular weights varying from 61.5 to 5.2 kDa. Previous studies, using one-dimensional SDS-PAGE, identified two bands (67 and 58.6 kDa), which were positively correlated with some semen parameters (sperm motility, sperm vigor, percentage of morphologically normal sperm and plasma membrane integrity). The 61.5 kDa band detected in the present study apparently corresponded to the 58.6 kDa band identified previously. Canine seminal plasma contained HBP; since HBP modulate the acrosome reaction in other species, they may have the same function in the dog. Further studies are necessary to better characterize this protein and determine if it is associated with fertility in the dog.

Keywords: Heparin-binding proteins; Seminal plasma; Dog; Canine

1. Introduction

The spermatozoon has capability to bind to heparin and to similar molecules, e.g. glycosaminoglycans (GAGs) in the oviduct. This capability to bind 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 [1]. Heparin-binding proteins (HBP) have been identified in several species, e.g. bovine [2], equine [3,4] and swine [5]. In dogs, the high degree of affinity between arginine esterase from seminal plasma and an equine HBP (SSP-7) has been described [6]. The objective of this study was to separate the HBPs from canine seminal plasma using heparin affinity chromatography.

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2. Materials and methods

2.1. Drugs and reagents

Chemicals and biochemicals were of the highest purity grade available and were purchased from the following companies: canine brucellosis test from Canine Brucellosis Antibody Test Kit, D-Tec® CB (Synbiotics Corporation, San Diego, CA, USA), vaccines from FortDodge (Duramune DA2PP + CvK/LCI® and Rai-Vac I®, Campinas, SP, Brazil) and ivermectin from Merial (Ivomec®, Campinas, SP, Brazil). All chemicals used for the electrophoresis and chromatography were purchased from Amersham Biosciences (Uppsala, Sweden), Sigma (St. Louis, MO, USA) or Merck S.A. (São Paulo, SP, Brazil).

2.2. Animals

In this study, three healthy crossbred dogs of unknown fertility were used. The dogs were housed in concrete-floored kennels with access to outside runs, water ad libitum and fed a commercial dog food (Pitukão®, Nutriara, Arapongas, PR, Brazil). The dogs had been tested for brucellosis and leptospirosis (microscopic serum-agglutination, Department of Infectious Diseases, UNESP, Botucatu, SP, Brazil), vaccinated against rabies, canine distemper, hepatitis, parainfluenza, laryngotracheitis, parvovirus, coronavirus and leptospirosis (L. canicola and L. icterohaemorrhagiae) infection and treated for external and internal parasites.

2.3. Semen sampling and processing

After 30 days of conditioning to semen collection (8–10 times), on six occasions ejaculates were obtained (by digital manipulation) from each of the dogs and pooled. Seminal plasma was separated from spermatozoa by centrifugation at 4200 × *g* for 1 h at 4 °C (MLW, K23, Engelsdorf, Leipzig, Germany). The heparin-binding proteins were isolated by heparin affinity chromatography (Äkta™ Oligopilot, Amersham Biosciences) as described by Manásková et al. [7] and modified as follows: the pooled seminal plasma (0.5 mL) was added to a heparin affinity chromatography column (16 mm × 26 mm, 1.0 mL/min) and equilibrated with PBS. Non-adsorbed proteins were washed out with PBS and bound proteins were eluded with 1 M NaCl. The data and graphics of the separated proteins in the chromatography were plotted by specialized software (UNICORN™, Amersham Biosciences). The recovered heparin-binding protein fractions were pooled. One-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) 12 and 18% were performed in vertical minigels (Mini VE®, Amersham Biosciences), as described by de Souza [8]. The gels were stained with silver nitrate, scanned (Image VDS, Amersham Biosciences) and molecular weight (kDa) values for each band within a lane were calculated with image analysis software (Image Master®, Amersham Biosciences).

3. Results

Fig. 1 shows the graphic image plotted on the heparin affinity chromatography. The electrophoresis analysis of the pooled eluded fractions identified 19 bands in two gel concentrations (12 and 18%), with molecular weights ranging from 61.5 to 5.2 kDa (Fig. 2). These bands are represented by the highest curve of Fig. 1, whereas the lowest curve (Fig. 1) represents proteins not bound to heparin.

4. Discussion

Previous studies using unidimensional polyacrylamide gel electrophoresis detected 37 bands in canine seminal plasma [8]. In the present study, 19 bands bound heparin. Thus, approximately 50% of canine seminal plasma bands bound heparin; and any of these could be involved in the acrosome reaction. Moreover, de Souza [8] also identified two bands (67 and 58.6 kDa) that were positively correlated to seminal parameters (e.g. sperm motility, sperm vigor, percentage of morphologically normal sperm and plasma membrane integrity). In the present study, a band with 61.5 kDa apparently corresponded to the 58.6 kDa band previously identified [8]. Earlier studies demonstrated a close relationship between HBPs and the acrosome reaction [1]. The present study identified this kind of proteins in canine seminal plasma. However, no relationship with fertility was determined. These proteins, especially the 61.5 kDa protein that is a HBP, could be involved in the canine acrosome reaction.

Five classes of HBPs have been described in the bovine [9]. Although all HBPs may bind to sperm surfaces [10], only one has been correlated with a greater fertility potential [11]. Moreover, these proteins were correlated with fertility potential after cryopreservation, which dramatically decreased (70–80%) the HBPs of bovine seminal plasma [12]. Thus, HBPs have been indicated as a biochemical marker to predict the fertility potential of bulls [11], and a commercial test

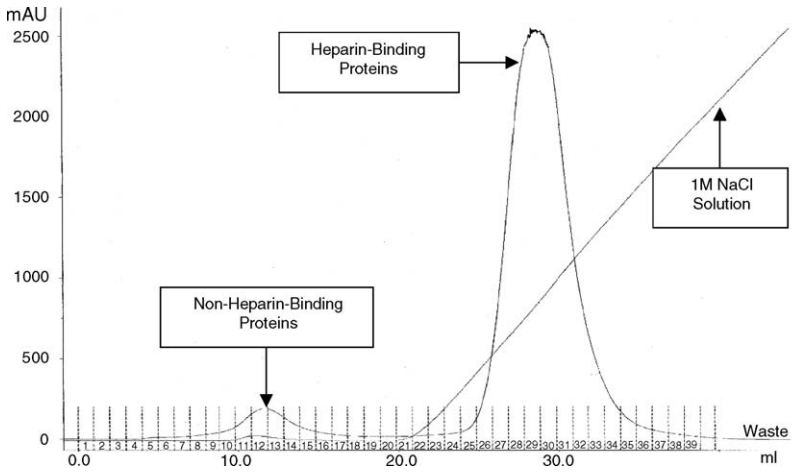

Fig. 1. Graphic image plotted by UNICORN Control System software (Amersham Biosciences, Uppsala, Sweden) of heparin-binding and non-heparin-binding proteins of canine seminal plasma separated on heparin affinity chromatography.

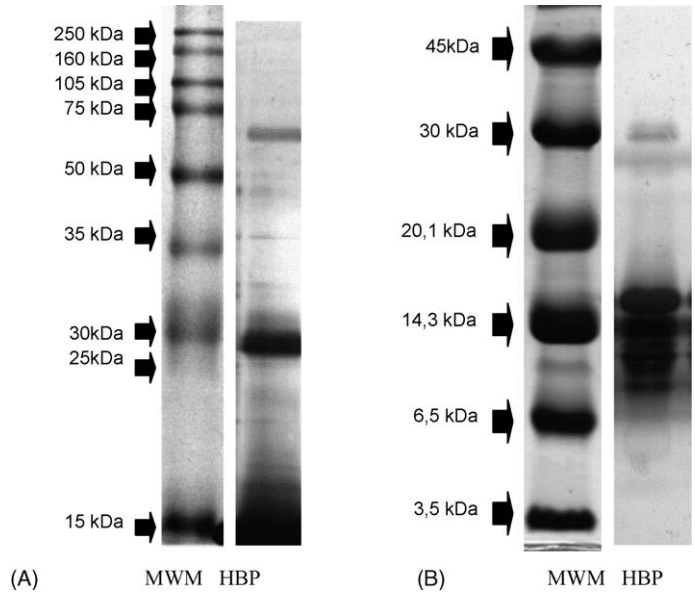

Fig. 2. Polyacrylamide electrophoresis gel (SDS-PAGE) at 12% (A) and 18% (B) in a discontinuous alcaline system of eluded fractions of heparin-binding proteins (HBP) of canine seminal plasma. The MWM indicates the molecular weight marker.

can be used to measure the presence of a protein on bovine sperm which corresponds to higher fertility (ReproduTest for Bulls, ReproTec Inc., Tucson, AZ, USA; www.reprotec.us). Whether the 61.5 kDa band is a biochemical marker for canine fertility or if it decreases following semen cryopreservation, remain to be determined.

In this study, the band B20 (15.6 kDa), a band with a higher concentration as demonstrated by densitometry (intensive stain in the gel), could be one of the arginine esterase subunits. The arginine esterase is a proteolytic enzyme that represents 90% of the total of canine prostatic fluid proteins [13,14]. Although approximately 50% of the canine seminal proteins bound to heparin, they represented the highest curve in the graphic image plotted on the heparin affinity chromatography, probably due to the great quantity of arginine esterase present in this fraction. This protein has a

molecular weight of 30 kDa and, upon denaturating conditions, it forms two subunits: H and L, 15 and 12–14 kDa, respectively [13,14]. Despite recent advancement in our knowledge of the biochemistry of this constituent [15–17], its biological function still remains a matter of speculation.

Calvete et al. [6] described the high degree of affinity between arginine esterase from canine seminal plasma and equine HBP (SSP-7). A study comparing the physicochemical characteristics of the canine arginine esterase and human prostatic specific antigen (PSA) from seminal plasma found high homology [18]. PSA has been identified as a HBP and is considered a human prostatic disease marker [19].

Seminal plasma is a complex fluid containing a wide variety of constituents, with proteins representing an important fraction. Characterization of seminal plasma proteins and studying their binding properties will be the first step in understanding their role in the fertilization process [20]. Since the HBPs were associated with fertility due to their modulatory role during the acrosomal reaction [1], their identification in the dog provides information that improves the understanding of physiological processes in this species. Moreover, the identification of such proteins in seminal plasma could provide better insight into the nature of subfertility or infertility in the dog, as well as enhance cryopreservation strategies. Further studies are needed to characterize these proteins and to elucidate their role in canine fertilization.

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