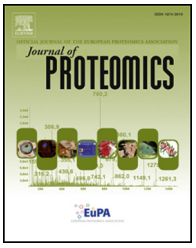
JOURNAL OF PROTEOMICS XX (2013) XXX–XXX

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot



Proteomic characterization and cross species comparison of mammalian seminal plasma

X. Druart[a,b,c,d,*], J.P. Rickard[f], S. Mactier[g], P.L. Kohnke[g], C.M. Kershaw-Young[f], R. Bathgate[f], Z. Gibb[f], B. Crossett[g], G. Tsikis[a,b,c,d], V. Labas[a,b,c,d,e], G. Harichaux[a,b,c,d,e], C.G. Grupen[f], S.P. de Graaf[f]

[a]INRA, UMR 85 Physiologie de la Reproduction et des Comportements, F-37380 Nouzilly, France
[b]CNRS, UMR7247, F-37380 Nouzilly, France
[c]Université François Rabelais de Tours, F-37000 Tours, France
[d]IFCE, F-37380 Nouzilly, France
[e]INRA, Plate-forme d'Analyse Intégrative des Biomarqueurs, Laboratoire de Spectrométrie de Masse, F-37380 Nouzilly, France
[f]Faculty of Veterinary Science, The University of Sydney, NSW 2006, Australia
[g]School of Molecular Bioscience, The University of Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 8 February 2013
Accepted 18 May 2013

Keywords:
Seminal plasma
Male fertility
Zinc alpha glycoprotein
Camelids
Ovulation
Nerve growth factor

ABSTRACT

Seminal plasma contains a large protein component which has been implicated in the function, transit and survival of spermatozoa within the female reproductive tract. However, the identity of the majority of these proteins remains unknown and a direct comparison between the major domestic mammalian species has yet to be made. As such, the present study characterized and compared the seminal plasma proteomes of cattle, horse, sheep, pig, goat, camel and alpaca. GeLC–MS/MS and shotgun proteomic analysis by 2D–LC–MS/MS identified a total of 302 proteins in the seminal plasma of the chosen mammalian species. Nucleobindin 1 and RSVP14, a member of the BSP (binder of sperm protein) family, were identified in all species. Beta nerve growth factor (bNGF), previously identified as an ovulation inducing factor in alpacas and llamas, was identified in this study in alpaca and camel (induced ovulators), cattle, sheep and horse (spontaneous ovulators) seminal plasma. These findings indicate that while the mammalian species studied have common ancestry as ungulates, their seminal plasma is divergent in protein composition, which may explain variation in reproductive capacity and function. The identification of major specific proteins within seminal plasma facilitates future investigation of the role of each protein in mammalian reproduction.

Biological significance
This proteomic study is the first study to compare the protein composition of seminal plasma from seven mammalian species including two camelid species. Beta nerve growth factor, previously described as the ovulation inducing factor in camelids is shown to be the major protein in alpaca and camel seminal plasma and also present in small amounts in bull, ram, and horse seminal plasma.

* *Corresponding author at:* UMR 6175 INRA, CNRS-Université de Tours-Haras Nationaux, Station de Physiologie de la Reproduction et des Comportements, Institut National de la Recherche Agronomique, 37380 Nouzilly, France. Tel.: +33 2 47 42 79 49.
E-mail address: Xavier.Druart@tours.inra.fr (X. Druart).

1874-3919/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jprot.2013.05.029

1. Introduction

Seminal plasma is a complex secretion of inorganic ions, sugars, organic salts, lipids, enzymes, prostaglandins, proteins and various other factors produced by the testes, epididymides and accessory sex glands (prostrate, vesicular, ampulla and bulbourethral glands) of the male [1]. While the true role of seminal plasma in sperm function and male fertility has been widely disputed, it is clear that this fluid aids in the transport of spermatozoa through both the male and female reproductive tracts while simultaneously influencing sperm physiology. For example, components within seminal plasma, particularly proteins, have been shown to influence sperm maturation [2,3], sperm membrane stabilization and capacitation [4,5] and even interaction with the oviduct [6,7] and oocyte [8]. Nonetheless, information on the effect of seminal plasma on sperm physiology is often contradictory, with seminal plasma reported to exert positive or negative effects on sperm function depending on the species studied. Even within the same species, huge variation in the effect of seminal plasma on sperm function has been described [9–11].

It has been hypothesized that this variation in function and effect could be explained by variation in the protein composition of seminal plasma, perhaps caused by the marked differences in accessory sex gland size and structure between the species. For example, the boar has very large bulbourethral, prostate and vesicular glands, while in the ram and bull the vesicular glands are still large but the bulbourethral and prostate glands are relatively small or disseminated [1]. In camelids, the vesicular glands are completely absent [12,13]. Unfortunately, this hypothesis remains untested as despite it having been long established that the inorganic composition of seminal plasma varies widely between species [14], variation in protein composition remains largely unknown. Among the seminal plasma proteins, the spermadhesins and the BSPs (binder-of-sperm-protein) have been extensively studied over the last years given their impact on sperm physiology and preservation [8,15–18]. But to date, a limited number of studies performing a systematic analysis of seminal plasma proteins using high throughput proteomics have been performed [19–21]. In fact, while the proteome of human seminal plasma has been comprehensively described with an actual list of more than 2000 proteins identified [22–24], relatively few of the proteins present within the seminal plasma of the major domestic mammalian species have been identified. This dearth of information is primarily due to the fact that global proteomics are yet to be applied in these species. Clearly, their application in a large-scale comparative study has the potential to greatly inform our understanding of the function of seminal plasma. Improved knowledge of the seminal plasma proteome would aid in the identification of those proteins responsible for reproductive functions specific to particular species e.g. induction of ovulation in camelids [25,26], as well as identify highly conserved seminal plasma proteins which may be essential to reproductive processes in all species. Candidate proteins to improve sperm function during application of assisted reproductive techniques such as cryopreservation or sex-sorting by flow cytometry may also be identified [27,28].

As such, the aim of the present study was to characterize and compare the seminal plasma proteomes of the main commercially relevant domestic mammalian species (pig, boar, bull, ram, buck, stallion, alpaca and camel) using GeLC–MS/MS and shotgun proteomic approach (2D–LC coupled with tandem mass spectrometry).

2. Methods

Procedures herein were approved by The University of Sydney's Animal Ethics Committee. Unless otherwise stated all chemicals were supplied by Sigma-Aldrich, NSW Australia.

2.1. Collection and preparation of seminal plasma

Ram (n = 3 males; Merino), bull (n = 12 males; Holstein), goat buck (n = 3 males; Alpine), camel (n = 3 males; Dromedary), horse (n = 3 males; Palouse) and alpaca (n = 3 males; Huacaya) semina were collected using artificial vaginae. Boar semen (n = 3 males; Large White) was collected using the gloved hand technique. Semen from each species was pooled and seminal plasma was separated from spermatozoa by centrifugation (10,000 ×*g*, 10 min, room temperature). The supernatant was centrifuged again (10,000 ×*g*, 10 min, room temperature) and stored at −80 °C.

2.2. SDS PAGE and densitometric quantification

SDS-PAGE electrophoresis was carried out according to Laemmli's method [29] on 8–16% gradient polyacrylamide gels (14 × 16 cm or 6 × 8 cm) using 15 μg of protein in each lane. After electrophoresis, proteins were Coomassie blue-stained and quantified. Densitometric quantification of Coomassie blue-stained protein bands was performed by transmission acquisition with an ImageScanner (GE Healthcare, Orsay, France) and analyzed with TotalLab (Nonlinear Dynamics Limited, Newcastle, UK). All values were normalized to a total volume of 100 and compared by calculating the average and standard error for three replicates.

2.3. GeLC–MS/MS

Proteins contained in the major bands of seminal plasma and observed after SDS PAGE and Coomassie staining were identified by tandem mass spectrometry (GeLC–MS/MS). The gel bands were cut into small blocks. Gel blocks were rinsed with water and acetonitrile before being reduced with 10 mM TCEP at 37 °C for 1 h and alkylated with 50 mM iodoacetamide for 30 min at room temperature in the dark. They were incubated overnight at 37 °C in 25 mM NH_4HCO_3 with 12.5 ng/μl trypsin (Promega, Sydney, Australia). The tryptic fragments were extracted, dried, reconstituted with 0.1% (v/v) formic acid, and sonicated for 10 min. They were then subjected to positive ion nano-flow electrospray analysis using a QSTAR Elite MS/MS instrument (Applied Biosystems/MDS SCIEX, Forster City, CA) coupled to a model 1100 capillary and nanoLC chromatographer (Agilent technologies, Palo Alto, CA). Samples were loaded on a reverse phase (RP) trapping cartridge (ZORBAX 300SB-C18 column — 0.3 × 5 mm, 5 μm particle size, 300 Å pore size) and separated using an analytical column (ZORBAX 300SB-C18 column — 0.1 × 150 mm, 3.5 μm particle size, 300 Å pore size).

Please cite this article as: Druart X., et al, Proteomic characterization and cross species comparison of mammalian seminal plasma, J Prot (2013), http://dx.doi.org/10.1016/j.jprot.2013.05.029