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Ram seminal plasma proteome and its impact on liquid preservation of spermatozoa

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ABSTRACT

Seminal plasma is composed of secretions from the epididymis and the accessory sex glands and plays a critical role in the fertilising ability of spermatozoa. In rams, analysis of seminal plasma by GeLC–MS/MS has allowed the identification of more than 700 proteins, including a high abundance of Binder of Sperm family proteins (BSP1, BSP5, SPADH1, SPADH2), the spermadhesin family (bodhesin2), lactoferrin and newly identified proteins like UPF0762 (C6orf58 gene). When spermatogenesis was stopped by scrotal insulation, changes in the proteome profile revealed the sperm origin of 40 seminal proteins, such as glycolysis pathway enzymes, the chaperonin containing TCP1 (CCT) complex and the 26S proteasome complex. Sperm mobility after liquid preservation (24 h in milk at 15 °C) is male dependent and can be correlated to differences in the seminal plasma proteome, detected by spectral counting. The negative association of zinc alpha-2 glycoprotein (ZAG) with semen preservation was confirmed by the use of recombinant human ZAG, which induced an increase in mobility of fresh sperm, but then decreased sperm mobility after 24 h of incubation. Several sperm membrane proteins interacting with the cytoskeleton, glycolysis enzymes and sperm-associated proteins involved in capacitation correlated with better liquid storage and can be considered as seminal biomarkers of sperm preservation.

Biological significance
Extensive analysis of the ram seminal plasma proteome reveals a complex and diverse protein composition. This composition varies between males with different sperm preservation abilities. Several proteins were shown to originate from the spermatozoa and positively correlate with sperm liquid preservation, indicating that these proteins can be traced as sperm

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biomarkers within the seminal plasma. The zinc alpha-2 glycoprotein (ZAG) was found to have a biphasic effect on sperm mobility, with a short-term stimulation followed by a long-term exhaustion of sperm mobility after a 24 h preservation period.

1. Introduction

During ejaculation, spermatozoa from the tail of the epididymis mix with secretions from the accessory sex glands (prostate, vesicular, ampulla and bulbourethral glands). Collectively known as seminal plasma, these secretions were initially thought to act merely as a transport medium for spermatozoa, but have since been recognised as a substance with a profound influence over sperm physiology and other reproductive processes [1,2]. The seminal plasma is composed of inorganic ions, sugars, organic salts, lipids, enzymes, prostaglandins, proteins and various other factors. Recent proteomic studies using high resolution strategies have provided extensive information about the human seminal plasma proteome [3–5]. These data now allow the use of the seminal plasma proteome as a tool to identify biomarkers of reproductive function [6]. In domestic animal species, the seminal plasma proteome has also raised interest, given its expected involvement in male fertility after the sperm preservation process [7,8]. The sheep is an interesting animal model for studying the role of the seminal plasma proteome in fertility, with previous data showing a positive effect of seminal proteins on sperm functions [9–11]. Several major proteins have been identified and quantified in ram [12–16] but comprehensive analysis of seminal plasma is needed to clarify the role of seminal plasma proteins. The aim of the current study was to (1) extensively describe the proteome of ram seminal plasma, (2) identify seminal proteins originating from sperm, (3) characterise inter-male variation of sperm liquid preservation ability and (4) discover seminal plasma proteins involved in liquid sperm preservation.

2. Materials and methods

2.1. Chemicals

Primary antibodies directed against the following proteins were purchased from Santa Cruz Biotechnologies (Santa Cruz, California): zinc alpha glycoprotein (dilution 1/500, sc-11358), 26S proteasome (1/100, sc-73488), lactoferrin (1/500, sc-52694), glucose 6 phosphate isomerase (1/500, sc-33777), alpha enolase (1/500, sc-15343), and TCP1 zeta (CCT6A) (1/500, sc-271734). Primary antibodies directed against the following proteins were purchased from Abcam (Cambridge, England): gelsolin (1/1000, ab11081), valosin containing protein (1/2000, ab11433) and heat shock protein 90 beta (1/1000, ab82522). Primary antibodies against angiotensin converting enzyme (ACE) and acrosin were produced from our laboratory after immunization of rabbits with purified ACE and acrosin. The second antibody was goat anti rabbit HRP (1/5000, A6154, Sigma) for primary rabbit antibodies and goat anti mouse HRP (1/5000, 172-1011, Biorad) for primary mouse antibodies. The chemiluminescent HRP substrate was SuperSignal West Pico and West Femto Chemiluminescent Substrate (Thermo Scientific, Waltham, USA). Recombinant human zinc alpha glycoprotein (4764-ZA) was purchased from R&D Systems (Minneapolis, MN). SYBR14 and propidium iodide were purchased from Life Technology (LIVE/DEAD® sperm viability kit).

2.2. Semen collection

All the procedures involving animals follow the recommendations of welfare from the Ministry of French Agriculture. Rams from Charollais, Texel, Suffolk and Vendéen breeds were housed at the Animal Insemination (AI) Centre (InsemOvin, Limoges, France) and Lacaune rams at the INRA Experimental Unit (Nouzilly, France). Ejaculates were collected using an artificial vagina following the standard procedure [17]. Only those ejaculates with an initial wave motion score of 4 or above were processed for liquid storage.

2.3. Scrotal insulation

Spermatogenesis of three Lacaune rams was stopped by a 2 °C elevation of the scrotum temperature using an insulated bag [30]. The bag was maintained in place continuously, and the animals were kept in a room with controlled light cycle and temperature (20–25 °C). The decrease in sperm production was ascertained by the total number of sperm in ejaculates collected as described above. After collection, seminal plasma was separated from sperm and/or cellular debris by centrifugation (two cycles at 18,000 ×*g*, 10 min, 4 °C), and the last supernatant was stored at −80 °C until use. Protein composition of seminal plasma was assessed by GeLCMS (gel-based liquid chromatography–mass spectrometry) which includes SDS-PAGE and in gel digestion followed by nanoLC–MS/MS, before scrotal insulation (normal sperm concentration; Control) and when no sperm could be found in the semen (Insulated).

2.4. Assessment of ram semen preservation ability

Rams were assessed for their ability to produce semen with a high preservation ability (HPA) or a low preservation ability (LPA) after 24 h of storage in a liquid state at 15 °C. Three ejaculates from 18 rams from the AI centre were diluted to 1.4×10^9 spermatozoa/ml with milk (reconstituted with 11.1 g of skim milk powder in 100 ml of distilled water and heated to 95 °C for 10 min) supplemented with gentamycin (50 μg/ml). A fixed volume of 1 ml of diluted semen was transferred into 1.5 ml tubes, placed in an incubator and cooled to 15 °C over the course of 3 h and stored at this temperature. Motility characteristics were analysed after equilibration at 15 °C (time 0 h) and after 24 h of storage at 15 °C (time 24 h).

2.5. Preparation of seminal plasma from HPA and LPA rams

After collection by an artificial vagina, semen from HPA rams was pooled (n = 5 rams, 2 series of 2 ejaculates/ram) as was