Theriogenology 82 (2014) 697–707

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Seminal plasma proteins of adult boars and correlations with sperm parameters

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ARTICLE INFO

Article history:
Received 23 January 2014
Received in revised form 28 May 2014
Accepted 28 May 2014

Keywords:
Proteomics
Seminal plasma
Swine
Semen parameter

ABSTRACT

The present study was conducted to identify the major seminal plasma protein profile of boars and its associations with semen criteria. Semen samples were collected from 12 adult boars and subjected to evaluation of sperm parameters (motility, morphology, vitality, and percent of cells with intact acrosome). Seminal plasma was obtained by centrifugation, analyzed by two-dimensional SDS-PAGE, and proteins identified by mass spectrometry (electrospray ionization quadrupole time-of-flight). We tested regression models using spot intensities related to the same proteins as independent variables and semen parameters as dependent variables ($P \leq 0.05$). One hundred twelve spots were identified in the boar seminal plasma gels, equivalent to 39 different proteins. Spermadhesin porcine seminal protein (PSP)-I and PSP-II, as well as spermadhesins AQN-1, AQN-3 and AWN-1 represented 45.2 ± 8% of the total intensity of all spots. Other proteins expressed in the boar seminal plasma included albumin, complement proteins (complement factor H precursor, complement C3 precursor and adipsin/complement factor D), immunoglobulins (IgG heavy chain precursor, IgG delta heavy chain membrane bound form, IgG gamma-chain, Ig lambda chain V-C region PLC3, and CH4 and secreted domains of swine IgM), IgG-binding proteins, epididymal-specific lipocalin 5, epididymal secretory protein E1 precursor, epididymal secretory glutathione peroxidase precursor, transferrin, lactotransferrin and fibronectin type 1 (FN1). On the basis of the regression analysis, the percentage of sperm with midpiece defects was related to the amount of CH4 and secreted domains of swine IgM and FN1 ($r^2 = 0.58$, $P = 0.006$), IgG-binding protein ($r^2 = 0.41$, $P = 0.024$), complement factor H precursor ($r^2 = 0.61$, $P = 0.014$) and lactadherin ($r^2 = 0.45$, $P = 0.033$). The percentage of sperm with tail defects was also related to CH4 and secreted domains of swine IgM and FN1 ($r^2 = 0.40$, $P = 0.034$), IgG-binding protein ($r^2 = 0.35$, $P = 0.043$) and lactadherin ($r^2 = 0.74$, $P = 0.001$). Sperm motility, in turn, had association with the intensities of spots identified as lactadherin ($r^2 = 0.48$, $P = 0.027$). In conclusion, we presently describe the major proteome of boar seminal plasma and significant associations between specific seminal plasma proteins and semen parameters. Such relationships will serve as the basis for determination of molecular markers of sperm function in the swine species.

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http://dx.doi.org/10.1016/j.theriogenology.2014.05.024

1. Introduction

Artificial insemination (AI) is the most important reproductive biology tool for genetic improvement aiming high prolificacy in pig industry [1,2]. The most successfully used germoplasm material in AI is the chilled semen, because boar spermatozoa cryopreservation procedures promote damages in sperm membranes and organelles, which in turn lead to lower fertility rates [1]. The knowledge of factors or substances that stabilize or protect boar spermatozoa for a successful fertilization process is crucial for animal production. This is the reason for the large number of researches in the area that aim not only to define good biological markers for the reproductive potential of boar, but also the development of products to be used as additive for the improvement of the present assisted reproduction methods.

In this context, seminal plasma is a promising source for the study of such potential biomarkers, because it is a complex mixture of secretions from testis, epididymis, and male accessory sex glands. In fact, the addition of 50% of boar seminal plasma to thawed sperm has been found to have benefic effects on the physiology of that cell [3]. Seminal plasma proteins, among many other substances present in the seminal fluid, are numerous and possess the ability to bind to the sperm during ejaculation, influencing several sperm criteria such as motility, capacitation, sperm transport, survival and longevity, protection against damages, and the formation of the sperm reservoir inside the female reproductive tract [4–6]. Given these multifunctional attributes of the seminal plasma components, the present study was conducted to identify the major seminal plasma protein profile of adult boars and also to evaluate its associations with several semen criteria.

2. Materials and methods

2.1. Animals and materials

The experiment was conducted at a commercial swine farm in the Northeast of Brazil (Maranguape, CE; 03° 54′ 46″ S, 38° 39′ 19.8″ W; Xerez Avicola, Ltda). Twelve sexually mature boars, routinely used as semen donors for AI, were housed in individual stalls (3.1 × 2.5 m) equipped with cooling systems. Animals were fed 2 kg/day with a commercial diet according to the nutritional requirement guidelines for adult boars and received water *ad libitum*.

Chemicals and equipment used in this work were purchased from Sigma-Aldrich (St. Louis, MO, USA), GE Life sciences (Piscattaway, NJ, USA), Bio-Rad (Rockville, MD, USA), Promega (Madison, WI, USA), Eppendorf (Hauppauge, NY, USA), and Waters (Milford, MA, USA).

2.2. Semen evaluation

Semen samples were collected by the gloved hand technique [7]. Aliquots of 20 μL were placed over glass slides and covered with cover slips, warmed at 37 °C, and subjected to light microscopy (200×) to evaluate the percentage of motile sperm [8] and sperm wave motion in a scale of scores from 0 to 5 [9]. Sperm concentration was determined by the Neubauer chamber. Sperm acrosome status was evaluated by the Trypan blue and Giemsa staining method [10]. Sperm cells were then visualized by light microscopy (1000×) and grouped as cells with intact or damaged acrosome.

Sperm morphology was evaluated by fixing the cells in a 1% (wt/vol) formaldehyde in PBS solution and evaluated under light microscopy (1000×), according to a previously described method [11], counting 200 cells per ejaculate. The integrity of the sperm plasma membrane was evaluated by the hypo-osmotic swelling test. Briefly, we incubated 100 μL of semen mixed with 1 mL of 100 mOs/kg distilled water for 40 minutes at 37 °C. After incubation, cells were fixed in a 1% formaldehyde solution. One aliquot (20 μL) of the mixture was mounted on glass slide and covered with cover slip and subjected to light microscopy (1000×). In this case, we counted 200 cells per ejaculation and evaluated the percentage of sperm with functional or damaged membranes, characterized by straight or coiled tail, respectively [12].

2.3. Two-dimensional electrophoresis of seminal plasma proteins

After ejaculation and separation of aliquots for sperm evaluation, the remaining semen samples were immediately mixed with a protease inhibitor cocktail containing 4-(2-aminoethyl) benzenesulfonyl fluoride, pepstatin A, E-64, bestatin, leupeptin, and aprotinin. Ten microliters of the cocktail was used per 1 mL of semen [13]. Boar semen was then centrifuged at 800× *g* for 15 minutes at 4 °C to separate the sperm cell. The supernatant seminal fluid was placed into a new tube and centrifuged at 5000× *g* for 60 minutes at 4 °C. The resulting supernatant was aliquoted and stored at −20 °C until use.

An aliquot of each sample of seminal plasma was thawed at room temperature and quantified as the total protein concentration [14] using bovine serum albumin as a standard. The assay was performed in triplicates. Then, a volume from each sample of seminal plasma containing 750 μg of total protein was mixed with hydration buffer (8 M urea, 1 M thiourea, 2% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate, 2% nonlinear ampholytes in the pH range 3–10, 25 mM dithiothreitol [DTT], and traces of bromophenol blue) sufficient to make 450 μL. These mixtures were then incubated with 24 cm immobilized pH gradient (IPG) strips (pH 3–10, nonlinear) in individual reswelling tray channels for approximately 20 hours. Isoelectric focusing was carried out in Ettan IPGphor 3 apparatus at 20 °C according to the following program: 200 V (60 minutes), 1000 V (60 minutes gradient), 7000 V (30 minutes gradient), 7000 V (60,000 Volt hours [Vh]) e 100 V (12 hours and 30 minutes), with a total of 16.300 Vh. After focusing, IPG strips were incubated (15 minutes) in equilibration buffer I (6 M urea, 50 mM Tris-HCl, pH 8.8, 29.3% (v:v) glycerol, 2% SDS, and 1% DTT) and re-equilibrated for additional 15 minutes in equilibration buffer II (similar to equilibration buffer I, but containing 2.5% iodoacetamide instead of DTT). After equilibration, strips were placed on the top of homogeneous SDS-PAGE gels (12.5%), sealed with agarose (5% in SDS-PAGE running buffer), and run at 500 V with 40 mA per gel.