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REVIEW PAPER

Proteomics of boar seminal plasma – current studies and possibility of their application in biotechnology of animal reproduction[1]

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SUMMARY

Proteomics is critical to identify the properties and functions of proteins involved in the mechanism regulating the male reproductive tract function. This approach is important in male fertility assessment and clinical diagnosis of the physiological state of individual reproductive organs. Proteomics also provides a tool to understand the interactions of seminal plasma proteins with spermatozoa, which could provide a useful model for studying ligand-cell interaction occurring at the sperm cell surface. This review covers a selection of advances in the realm of functional proteomics of boar seminal plasma proteins and is focused on some fundamental proteomic technologies. Also, this review explores key themes in proteomics and their application in animal reproductive techniques. *Reproductive Biology 2005 5 (3):279-290.*
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INTRODUCTION

After the determination of many complete prokaryotic and eukaryotic genome sequences, the elucidation of gene/protein function is the next big challenge in biotechnology. The term "proteomics" can be defined as the qualitative and quantitative comparisons of proteomes to identify cellular mechanisms which are involved in biological processes [8].

The strategy used to analyze the mammalian proteome has shown that proteomics is not only based on the identification and quantification of proteins, but also is the study of their structure, localization, modification, interactions, activities and functions [30]. Hitherto, no method has been developed with a resolution greater than two-dimensional (2-D) polyacrylamide gel electrophoresis, which is the cornerstone technique of proteome analysis. The differentially expressed proteins in response to changes in cellular states are especially focused and analyzed with the 2-D gel electrophoresis technique. This methodology termed "expression proteomics" worked as a major driving force behind proteomics analysis. However, the conventional 2-D electrophoresis only shows protein expression and cannot detect protein-protein interactions and protein function in principle, without using particular methods such as affinity chromatography or gel chromatography (multi–dimensional chromatography). Thus other approaches are required to give a comprehensive understanding of cellular mechanisms at the protein level.

Although proteomics traditionally dealt with quantitative analysis of protein expression, more recently, proteomics has been viewed to encompass function analysis of proteins. Functional proteomics is an emerging field of systematic protein analyses, which gives detailed information about protein-protein interactions and their biological function [32]. Here, we present an overview of the current status of some selected elements of functional proteomics of boar seminal plasma components, particularly those related to the basic physiologic function of spermatozoa in the reproductive processes of swine.

FUNCTIONAL PROTEOMICS OF BOAR SEMINAL PLASMA – BIOCHEMICAL APPROACHES

Spermadhesins - multifunctional proteins

Accumulating evidence has shown that the process of gamete recognition and sperm – egg binding is mediated by the type of carbohydrate protein interactions. Previous studies showed that zona pellucida of mammalian oocyte is composed of only two – four glycoprotein families, which seem to facilitate its defined biological function [27, 28]. The groups of sperm-coating proteins, which bind to the zona pellucida, are different in relation to their biochemical structure and molecular weights (14 to 90 kDa) and may vary from species to species.

Spermadhesins, a novel family of secretory proteins expressed in the male genital tract of the boar, stallion and bull, have been found to be peripherally associated with the sperm surface [29]. It has been shown that differentiation in the functional properties of spermadhesin is facilitated by post-translational modifications, especially glycosylation [5]. Moreover, it is of interest that glycosylation not only contributes to the structural diversity of the protein family, but also modulates the ligand-binding capabilities of the glycosylated spermadhesins, that is, it abolishes their zona pellucida-binding activity without impairing the heparin-binding ability [4, 5]. Spermadhesins are multifunctional proteins showing a wide range of ligand – binding abilities, for example, saccharides, sulfated glycosaminoglycans, phospholipids and proteinase inhibitors, suggesting that they are implicated in different stages at fertilization [7, 28].

It has been reported that, in the boar, the bulk of the seminal plasma proteins belong to the family of spermadhesins, which are synthesized mainly by the vesicular glands and, in some cases, by the epididymis and rete testis [4]. There is abundant literature regarding the structure and biochemical properties and physiological functions of boar spermadhesins. Seven spermadhesins, PSP-I, PSP-II, AQN-1, AQN-2, AQN-3, AWN and DQH, have been identified in boar seminal plasma and all of them, except PSP-I, show heparin – binding ability [6, 7]. It is noteworthy that under physiological conditions the

aggregated forms of sperm surface proteins (PSP, AQN, AWN and DQH) strongly predominate over their monomeric forms in boar seminal plasma. These interactions facilitate the formation of aggregated forms of proteins in the seminal plasma and probably the arrangement and remodeling of sperm coating proteins. It is interesting that the heparin-binding activity of aggregated forms of proteins from boar seminal plasma corresponds to the activity of isolated monomers with only one exception: heterodimer PSP-I/PSP-II spermadhesin, which displays carbohydrate binding activity linked to the PSP II subunit [6, 18]. Recently, it has been confirmed that PSP-I/PSP-II spermadhesin stimulates macrophages to release a neutrophil chemotactic substance, indicating the role of spermadhesin-heterodimer as a modulator of the uterine immune activity [1].

Boar seminal plasma proteins – role in reproductive function

Classically the 2-D gel electrophoresis, performed as a combination of isoelectric focusing and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), has been the only method used to analyze the proteome with high resolution. This method enables the separation of thousands of proteins that are expressed in a specific cell or a specific tissue [12]. The two-dimensional (2-D) gel electrophoresis method was utilized for the first time to study proteome of the boar reproductive tract [21].

The 2-D gel electrophoresis of boar seminal plasma showed a few major polypeptides from the cauda epididymal fluid, seminal vesicles, bulbourethral glands or prostate gland. It has been shown that numerous neutral and basic, low-molecular weight polypeptides originating from boar vesicular glands adhered tightly to the spermatozoa. It is interesting that the fluid of seminal vesicles contains about 270 polypeptides, while the epididymal fluid contains about 160 polypeptides, which migrate towards neutrality or in the highly basic region during 2-D gel electrophoresis.[1]

In our recent study, we have applied proteomics based on the use of 2-D gel electrophoresis to show that there are qualitative changes in the protein

[1] Kordan W, Soliwoda D, Strzezek J **2004** Polymorphism of seminal plasma proteins as a marker of male fertility. *Proceedings of the Twelfth Congress of the Polish Veterinary Society,* 15-17 September 2004 Warsaw, p. 68.

profiles of boar seminal plasma, in relation to the animal age[1]. Moreover, the use of affinity chromatography, with lectin concanavalin A (Con A), showed that a series of polymannose glycoproteins are predominant in boar seminal plasma [26]. This study demonstrated that numerous new acidic-neutral and basic range polypeptides (20 to 40 kDa), probably originating from the seminal vesicles (the dominant source of seminal proteins), were present in the seminal plasma of three year-old boars. These changes in the protein profiles suggest that the process of sexual maturation in the boar is synchronized with the glycosylation reactions accompanying protein secretions, which are more intensive for boars under 12 months of age. It should be noted that protein secretions in boar seminal plasma are also influenced by seasonal variations [26].

Generally, seminal plasma contains specific protein factors that influence both the fertilizing ability of spermatozoa and exert important effects on the female reproductive physiology. Seminal plasma proteins are involved in the control of molecular mechanisms accompanying sperm transport in the female reproductive tract, suppression of the immune response against sperm antigens and gamete interaction following egg fertilization. However, the development of *in vitro* fertilization techniques and embryo micromanipulation is dependent on answering many questions regarding the function of seminal plasma proteins in the fertilization process. It has been recently shown that pre-incubation of flow-sorted boar spermatozoa with seminal plasma increased their ability to penetrate IVM-oocytes [19]. Moreover, the regulatory action of seminal plasma proteins is manifested at different molecular events accompanying various stages of animal reproduction.

It has been shown that the seminal plasma has an important role in maintaining the optimal level of Zn^{2+} ions. Studies on the decondensation process of human spermatozoa have shown that the seminal vesicle secretes high-molecular weights zinc ligands that reduce zinc content in the sperm chromatin [14]. On the other hand, the prostatic fluid is rich in free Zn^{2+} ions or Zn^{2+} ions bound with low-molecular weight components,

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which may act as a modulator of sperm chromatin condensation. The basic modulator of the sperm chromatin condensation appears to be Zn^{2+} ions and their ligands originating from the vesicular glands of the boar [23, 24].

Accumulating evidence has been shown that the seminal plasma plays a pivotal role in the regulation of sperm motility. Recent advances concerning peptide inhibitors of sperm motility, which have been isolated from human and boar seminal plasma, have been remarkable. More extensive information on the effect of seminal plasma inhibitors on boar sperm motility is presented elsewhere [17]. It is noteworthy that these peptide inhibitors, originating in the seminal vesicle secretions, have similar structural homology with important spermadhesins such as AQN-3 and DQH of boar seminal plasma. This homology may indicate a role of plasma membrane receptors, at the region overlying the middle and tail pieces, in the regulation of mammalian sperm motility apparatus.

Boar spermatozoa are exceptionally susceptible to the action of reactive oxygen species (ROS) because they have a high concentration of polyunsaturated fatty acids in their plasmalemma and inadequate enzyme antioxidant system in their cytoplasm of the middle piece. The low levels of superoxide dismutase (SOD) and the lack of glutathione peroxidase (GPx) and catalase in the seminal plasma indicate that boar spermatozoa are poorly adapted to counteract the toxic effects of induced ROS. However, this is compensated by the pivotal role of the sulphur – containing antioxidants (L-glutathione and L-ergothioneine) and L-ascorbic acid as well as the high protein antiperoxidant activity of boar seminal plasma. Furthermore, seminal plasma proteins have been shown to be significant in the defence of spermatozoa against ROS. Vesicular Zn^{2+}-dependent protein of boar seminal plasma has been shown to possess antiperoxidant properties [25]. Besides the antiperoxidant properties of seminal plasma, immuno-modulation proteins play an important role in the suppression of formation of antisperm antibodies. For example, our previous studies on the boar showed that the heterogeneous 54 kDa glycoprotein of seminal plasma and vesicular fluid blocked spontaneous proliferation of lymphocytes [22]. This study also demonstrated that a protein fraction with molecular weight

of 15 kDa, which is a component of 54 kDa glycoprotein, showed a binding ability towards pig IgG.

In our laboratory we have used a combination of 2D SDS-PAGE and Western blot analysis, using specific polyclonal antisera, to detect proteins in the polypeptide profile of boar seminal plasma. These methods have enabled the identification of the molecular forms of protein tyrosine acid phosphatase (PTAPase), platelet activating factor acetylhydrolase (PAF-AH) and SOD (EC-SOD). Some of the biochemical properties of these enzymes, which have been purified in our laboratory, are shown in Table 1.

SOME APPLICATIVE ASPECTS OF PROTEOMICS OF SEMINAL PLASMA

Seminal plasma proteins as a marker of the fertilizing ability of boar semen

The biological effects of seminal plasma proteins on sperm function are complex and not fully understood. It is generally accepted that the binding of seminal plasma proteins to spermatozoa stabilizes the components of the plasmalemma, mask antigens exposed to cell surface and prevent a premature acrosome reaction. Seminal plasma components have been shown to elicit inflammatory response in the female reproductive tract, including altered patterns of cytokine secretions, which may be important for embryo development and implantation.

The presented functions of proteins have prompted researchers to search for biochemical markers of seminal plasma that can serve as diagnostic indicators of fertility potential. With the use of 2D electrophoresis, Flowers[1] showed the biological importance of the two proteins (26 kDa, pI 6.2; 55 kDa, pI 4.8) present in boar seminal plasma and claimed that high concentrations of both (relative units greater than 10) in boar ejaculate corresponded with high farrowing rates (more than 86%) and number

[1] Flowers WL **1998** Boar fertility and artificial insemination *Proceedings of the Fifteenth International Pig Veterinary Science (IPVS) Congress*, Birmingham, p. 45-52.

Table 1. Biochemical properties of enzymes purified from boar seminal plasma

Biochemical property	**Phosphotyrosine protein acid phosphatase (PTPase)**	**Platelet activating factor – acetylhydrolase (PAF-AH)**	**Superoxide dismutase (EC-SOD)**
Secretory source	Seminal vesicles	Seminal vesicles, Prostate	Epididymis, Seminal vesicles, Prostate
Purification factor (fold)	500	44	355
Structure	glycoprotein; one molecular form non-metalloprotein	glycoprotein; aggregate of four molecular forms	glycoprotein; one molecular form metalloprotein (Cu/Zn SOD)
Molecular weight	41 kDa	310 kDa	67 kDa
pI	7.1	4.6	8.8
Optimum pH	5.5	7.3	9.5
Thermal stability	high	high	high
Substrate affinity (high activity)	phosphotyrosine and tyrosine - phosphorylated oligopeptides	PAF and sperm plasmalemma	Dismutation of the superoxide anion
N-terminal sequence	Only 92% homology with the human and rat prostate acid phosphatase.	Molecular form of 43 kDa, similar to Ig – binding proteins and zona adhesion protein.	Not analyzed
Suggested functions	Enzyme is crucial in sperm function such as protein tyrosine phosphorylation, acrosome reaction and fertilization.	Regulates PAF levels, which control sperm motility, capacitation and acrosome reaction, and may act as a "decapacitation factor".	Enzyme is established in spermatozoa at the early stages of maturation. High SOD activity in seminal plasma plays a major role in protecting spermatozoa against reactive oxygen species.

Wysocki & Strzeżek [31]; Kordan [15]; Kordan et al. [16];

[1]Kuklińska M, Strzeżek J **2004** Superoxide dismutase (SOD) as a major antioxidant enzyme of boar semen. *Proceedings of the Twelfth Congress of the Polish Veterinary Society,* 15-17 September 2004 Warsaw p.69;

[2]Strzeżek J, Kosiniak-Kamysz K, Kuklińska M, Bittmar A, Podstawski Z, Rafalski G **2000** Enzymatic and non-enzymatic antioxidants in stallion and boar semen. *European Society on Domestic Animal Reproduction (ESDAR) Newsletter* 5 p. 56.

of piglets born alive (more than 11). Evidence has been shown that the inclusion of 10-12% of seminal plasma to an artificial insemination (AI) dose may have a beneficial effect on the biological value of the boar semen[1] [11]. Moreover, Killian et al [13] have shown that glycoproteins in seminal plasma differed among bulls varying from high to low non-return rates (two proteins: 26 kDa 6.2 pI; 55 kDa 4.5 pI predominated in higher- fertility bulls whereas two other proteins: 16 kDa 4.1 pI; 16 kDa 6.7 pI were more prevalent in below average fertility bulls). Moreover, besides the regulatory functions in the fertilization process, seminal plasma proteins play an important role in the suppression of uterine inflammatory response caused by rapid leucocytosis following deposition of boar semen in the sow reproductive tract [11, 20].

Accumulating evidence has been shown that seminal plasma proteins may have a role in preservation technology of boar semen [2, 3, 9, 10]. For instance, the supplementation of extended boar semen with purified samples the non-binding PSP–I/PSP–II spermadhesin had a beneficial effect on viability of the preserved spermatozoa [9]. However, recent studies have shown that the supplementation of freezing extenders with PSP-I/PSP-II spermadhesin did not affect post-thaw sperm survival, but had an inhibitory effect on the fertilizing ability of frozen-thawed boar spermatozoa [2, 10]. It is surprising that PSP–I/PSP–II spermadhesin does not bind to the sperm surface, thus excluding its role in gamete interaction. Nevertheless, the biological function of PSP–I/PSP–II spermadhesin on sperm function has not been fully elucidated yet.

Dialysis of boar semen – effects on polypeptide profile of the seminal plasma

Our research team has conducted experiments to determine whether a 5-h period of dialysis of boar semen with 12-14 kDa cut-off membrane would change the polypeptide profile of the seminal plasma and how it

[1] Flowers WL **1998** Boar fertility and artificial insemination. *Proceedings of the Fifteenth International Pig Veterinary Science (IPVS) Congress*, Birmingham, p. 45-52.

would influence post-thaw sperm quality. Our preliminary results showed that dialysis of boar semen had a significant effect on the polypeptide profiles of boar seminal plasma. Dialysis of boar semen changed both the isoelectric point and molecular weight of polypeptide profiles of seminal plasma of individual boars. Wide variations in the quantitative changes in the polypeptide profile, with isoelectric points less than 7.4 or more than 7.4 for dialyzed seminal plasma, were evident among boars. Furthermore, these changes were accompanied by enhanced post-thaw sperm quality, such as sperm motility, viability and mitochondrial activity. As a practical aspect, dialysis of semen prior to freezing may be a useful technique to optimize the quality of boar spermatozoa following thawing. However, in order to improve the sperm fertilizing ability further studies on the effects of dialysis on the sperm biological structures are warranted.

CONCLUSIONS

The physiological functions of a large number of seminal plasma proteins have not been fully elucidated, despite the fact they, at least in part, have been well characterized. This review shows that the potential impact for proteomics in molecular research in animal reproduction is enormous. Furthermore, the application of proteomic technologies can help uncover various changes in protein structure, function and protein-protein interactions, which can modulate sperm function, such as hyperactivation, capacitation, and acrosome reaction. It can be suggested that functional proteomics will contribute greatly to our understanding of the molecular basis of most aspects of seminal plasma protein function in the reproductive processes. This approach holds promises for assessment of male fertility assessment and monitoring changes in the reproductive tract of different animal species.

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