# BOAR SEMINAL PLASMA PROTEINS AND THEIR BINDING PROPERTIES. A REVIEW

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Binding properties of a group of proteins isolated from boar seminal plasma and their role in the fertilization process are discussed. Boar seminal plasma contains different types of proteins: spermadhesins of AQN and AWN family, DQH and PSP proteins belong to the most abundant. Some of these proteins are bound to the sperm surface during ejaculation and thus protein-coating layers are formed. Sperms coated with proteins participate in different types of interactions in the following steps of the fertilization process: formation of oviductal sperm reservoir, sperm capacitation, oocyte recognition and sperm binding. Saccharide-based interactions of boar seminal plasma proteins play role in the binding of sperm to oviductal epithelium, in sperm capacitation and primary binding of sperm to zona pellucida. An interaction with phospholipid components is responsible for the protein adsorption to sperm membrane. Interactions between proteins participate in the arrangement and remodelling of sperm-coating layers. Study of boar seminal plasma proteins, their characterization and elucidation of their interactions will contribute to understanding the fertilization process. A review with 82 references.

**Keywords**: Seminal plasma proteins; Boar seminal plasma; Binding properties of proteins; Fertilization; Sperm; Spermadhesins; Gametes.

## 1. INTRODUCTION

Mammalian fertilization is a unique event in which morphologically disparate gametes recognize each other, bind and fuse. This event includes highly regulated biochemical interactions between molecules located on the surface of both gametes as well as substances present in the natural environment of gametes both in the male and the female reproductive organs. The following phases can be distinguished: binding of seminal plasma proteins to the sperm surface during ejaculation, interaction of sperm surface proteins with oviductal epithelial cells, sperm capacitation, gamete recognition, primary and secondary binding of the sperm to the zona pellucida, acrosome reaction of sperm, penetration of the sperm through the zona pellucida of the ovum and fusion of the sperm and the egg (reviewed in literature[1–5]).

Seminal plasma proteins participate in events that occur both in the male and the female reproductive organs. Mammalian seminal plasma is a complex mixture of secretions originated from the testis, the epididymis, and the male accessory sex glands (seminal vesicles, ampulla, prostate, bulbourethral glands). Seminal plasma is a very complex fluid containing a wide variety of both organic and inorganic components, among which proteins are an important part of the high-molecular-weight substances. Proteins of seminal plasma are bound to the sperm surface during ejaculation and thus protein-coating layers are formed. This sperm coverage is rearranged during the following steps of fertilization. In the female reproductive organs seminal plasma proteins bound on the sperm surface most probably participate in formation of the oviductal sperm reservoir, sperm capacitation, oocyte recognition and sperm binding to the ovum[1,6].

The following interactions of sperm that are coated with seminal plasma proteins are among the most studied ones:

– the interaction with different types of glycoconjugates,
– the interaction with membrane phospholipids,
– interactions between proteins.

### *1.1. Saccharide-Based Interactions*

Saccharide chains linked to proteins or lipids occur in many biological systems. Glycoconjugates are present in the cell membrane, in components of both the extracellular fluids and the intracellular content. Saccharide moieties of these macromolecules exercise a variety of functions. Out of them, specific targeting of the macromolecular substances, in particular proteins,

belongs to the most important. The saccharide recognition mechanism is crucial in many biological processes, e.g. cell–cell adhesion, cell–matrix interaction, interactions between proteins, etc. The lectin-type interactions also participate in different phases of the fertilization process[4,6,7].

The formation of the sperm oviductal reservoir is one of the described saccharide-mediated events of the fertilization process in which, most probably, proteins adsorbed to the sperm surface participate. Information about this process has been obtained mostly from the competitive inhibition studies of sperm binding to explants of oviductal epithelium[8,9]. The saccharide specificity of sperm binding to epithelium differs for different species[4]. In the pig the formation of the sperm reservoir appears to involve the interaction of high-mannose type oligosaccharide chains[10,11].

Primary binding to zona pellucida glycoproteins is a further studied saccharide-mediated event of the fertilization process. It is well accepted that gamete recognition involves an interaction of exposed ligands of the oocyte extracellular matrix, the zona pellucida, with a set of receptor molecules of the sperm. The zona pellucida glycoproteins form a three-dimensional matrix with specifically oriented saccharide chains to operate the primary binding between the sperm and the egg. The first studies involved an investigation of inhibition of sperm-egg binding by different saccharides in numerous species (reviewed in literature[1,6]).

Saccharide chains of zona pellucida glycoproteins are assumed to bind receptors present on the sperm surface. Although the saccharide ligands were first characterized and studied in detail in mice[12] and pigs[13–16], the ligands of other mammals are not fully recognized. Proteins that are present on the sperm surface belong to candidates being responsible for the sperm–zona pellucida glycoprotein interaction. A number of them from different species were isolated and their ability to interact with zona pellucida glycoproteins has been described[16,17]; there exist uncertainties regarding the physiological role of this type of interaction.

### *1.2. Interactions with Membrane Phospholipids*

Proteins of seminal plasma are secreted mostly by seminal vesicles and they bind to the sperm surface at ejaculation. Protein interaction of seminal plasma proteins with components of the sperm membrane was thoroughly studied in the case of the bull model[18]. Various biochemical techniques have been used to show an interaction of bull seminal plasma (BSP) proteins with phosphatidylcholine, phosphatidylcholine plasmalogen and sphingomyelin[18,19]. Choline phospholipids account for more than 60% of

the total phospholipids of bull sperm membrane[20]; due to this fact phosphorylcholine was used as a model ligand for studies in this direction. Much less is known about the interaction of sperm-coating proteins with membrane phospholipids in other species, e.g. stallion[21] or boar[21–23].

### *1.3. Interactions Between Seminal Plasma Proteins*

Interactions between seminal plasma proteins have not yet been studied in detail. It is now evident that such a type of interaction participates in the formation of aggregated forms of seminal plasma proteins and protein coverage of the sperm surface. The composition of protein coating layers of sperm that are formed during ejaculation is changing in the course of the route of sperm to the ovum. An important remodeling of the protein coverage of the sperm surface occurs in the female reproductive organs. The existence of aggregated forms of proteins in seminal plasma has been described only for some species[18,24,25], but very little is known about the physiological role of such association and dissociation processes. The aggregation state of the main stallion seminal plasma proteins has been described to depend on the glycosylation degree[26].

The aggregation of bull seminal plasma proteins is one of the most studied cases. These proteins were described to be present in seminal plasma in a polydisperse form[27]; however, changes in the polydispersity in the course of the fertilization process are not fully understood. The aggregation state of the major protein of bull seminal plasma (PDC 109) could be modulated by substances of the native environment of gametes[28–30].

## 2. BOAR SEMINAL PLASMA PROTEINS

The role of seminal plasma proteins adsorbed on the sperm surface in all above-mentioned phases of the fertilization process is not fully understood and at present it is evident that it varies from species to species. Nevertheless, proteins of seminal plasma of different species so far investigated exhibit an ability to interact with a wide variety of ligand types and thus they affect the binding properties of sperm.

We have chosen the boar model to study the relationship between the properties of seminal plasma proteins that are adsorbed on the sperm surface during ejaculation, the binding properties investigated in vitro and a possible physiological role. Detailed knowledge of properties of the individual isolated proteins and of their binding properties with respect to model ligands represented the first step of the study. The next steps involved an

investigation of the state or a form in which these proteins are present under physiological conditions and binding studies with respect to more complex endogenous ligands.

### *2.1. Properties of Boar Seminal Plasma Proteins*

Boar seminal plasma contains different types of proteins; some of them belong to spermadhesins, a group of 12 000–16 000 polypeptides. Spermadhesins are a novel family of secretory proteins expressed in the male genital tract of boar, stallion and bull. They are major products of the seminal plasma and have been found to be peripherally associated to the sperm surface. They are multifunctional proteins showing a range of ligand-binding abilities, e.g. saccharides, sulfated glycosaminoglycans, phospholipids and proteinase inhibitors, suggesting that they may be involved in different steps of fertilization. AQN, AWN and PSP (porcine seminal plasma) proteins belong to the spermadhesin family. The N-terminal amino acid sequence of all members of AQN and AWN proteins begins with either Ala-Gln-Asn (AQN) or Ala-Trp-Asn (AWN). Spermadhesins (AQN, AWN, PSP proteins) are characterized by 40–60% amino acid sequence identity, contain two conserved disulfide bridges and belong to one protein family (reviewed in literature[7,16]). A sequence pattern-search analysis revealed that spermadhesins belong to a family of 16 functionally diverse proteins, many of which are known to be involved in developmental processes[32]. The structure of the proteins is built by a combination of several modules, all of which share the CUB domain (named after the proteins in which it was first identified: C, complement subcomponents C1r/C1s; U – uegf, urchin epidermal growth factor; B – Bmp1, bone morphogenetic protein 1). Spermadhesins form a subgroup of the CUB family, in which the presence of nine anti-parallel β-strands, like that observed in immunoglobulin domains, has been proposed.

Another type of protein, designated DQH (according to its N-terminal amino acid sequence) was isolated from boar seminal plasma and differs in its structure from boar spermadhesin. The DQH protein is homologous to major proteins of bull and stallion seminal plasma. Binding of the biotinylated DQH protein to the acrosome of boar epididymal spermatozoa indicates that this protein belongs to the sperm surface proteins[33]. The covalent structure of the DQH protein has been determined[34]. The protein is a polypeptide of 105 amino acids with four disulfide bridges in the molecule and consists of an N-terminal O-glycosylated peptide followed by two

fibronectin type II repeats. The DQH sperm surface protein is a member of the large family of cell and matrix adhesion proteins[21,33,34].

Proteinase inhibitors represent a further group of protein components of boar seminal plasma. In our first studies, several proteinase inhibitors have been isolated and characterized from the boar reproductive tract[35]. Out of them the best characterized are acrosin inhibitors, proteins structurally related to trypsin-like proteinase inhibitors of the Kazal type isolated from boar spermatozoal extracts and seminal plasma. The sequence of the inhibitor from the spermatozoa known as sperm-associated acrosin inhibitor (SAAI) was 90% identical to that of boar seminal plasma[36]. The isoforms of the inhibitor from seminal plasma are glycosylated[37], while those sperm-associated do not contain saccharides[36]. It is generally accepted that the biological role of acrosin inhibitors is to inactivate the prematurely released acrosin from occasionally damaged spermatozoa and thus to protect the male and the female genital tract against proteolytic degradation[38]. AQN, AWN spermadhesins and SAAI are secretory components of seminal vesicles that bind to sperm heads at ejaculation[39,40]. Spermadhesins attached to the sperm head are acceptor molecules for the SAAI inhibitor[36,41,42].

Besides spermadhesins, proteinase inhibitors, and DQH sperm surface protein, β-MSP and lactoferrin are also protein components of boar seminal plasma[43,44]. All the above mentioned proteins represent well-characterized molecules and their physicochemical properties are summarized in Table I. Almost all these protein components are present in seminal plasma under physiological conditions in the form of aggregates of different molecular weight, composition and binding properties[25,45,46] (Table II).

### *2.2. Saccharide-Binding Activity of Boar Seminal Plasma Proteins*

Studies on the saccharide-binding activity of boar seminal plasma proteins were aimed at the elucidation of their role in individual phases of the fertilization process. The choice of ligands was directly related to substances present in the natural environment of sperm both in the male and the female reproductive organs.

Proteins of boar seminal plasma were shown to interact with various types of saccharides and glycoconjugates. The following types of saccharides are among the most studied substances:

– heparin and other sulfated polysaccharides,
– hyaluronic acid,
– zona pellucida glycoproteins,

TABLE I
Proteins of boar seminal plasma and their physicochemical properties

| Protein | Structural characteristic | Mol. weight | N-terminal amino acid sequence | Isoelectric point | Isoforms | References |
|---|---|---|---|---|---|---|
| PSP I | spermadhesin | 14 000 | LDYHACCGCRLD.... | 7.82[b] | single N-glycosylation site | 78 |
| PSP II | spermadhesin | 14 000 | ARINGDECGRVI.... | 9.72[b] | single N-glycosylation site, heterogeneity in the oligosaccharide chain | 79, 80 |
| AWN 1 | spermadhesin | 16 000<br>17 000<br>14 000 | AWNRRSRSCGGVLRD....<br>RSRSCGGVLRDPP....<br>RSCGGVLRDPPGK.... | 9.0[b]<br>9.3[b]<br>9.3[b] | a) truncated forms from N-terminus<br>b) glycosylate isoforms (N-linked saccharide chains) | 33, 57, 65 |
| AWN 2 | spermadhesin | 16 000 | N-terminus blocked | 6.4[a] | | 57 |
| AQN 1 | spermadhesin | 13 000 | AQNKGPHK... | 9.0[b], 8.9[a] | | 33 |
| AQN 2 | spermadhesin | 14 000<br>16 000 | AQNKGSDDXD... | 9.3[b], 8.1[a],9.5[b], 8.9[a] | AQN2 is a glycosylated form of AQN3 | 33, 64 |
| AQN 3 | | 12 000 | | 9.4[b] | | |
| DQH | two fibronectin type II domains | 13 000 | DQHLPGRFLPAIT.... | 8.6[b], 8.6[a] | | 21, 33, 34 |
| β-MSP | microsemiprotein family | 10 026 | ZYFING.... | 8.07[b] | | 43, 81 |
| Serine proteinase inhibitors | seminal plasma inhibitor (SPI) | 12 000 | TRKQPNCNVYR.... | 9.5[b] | isoforms differ only at their N- and C-terminal parts | 37 |
| | sperm-associated acrosin inhibitor (SAAI) | 8 000 | ARSKKTRKEPDCDVYR.... | 8.3[b] | | 36 |
| Lactoferrin | transferrin family | 70 000<br>70 000 | <br>N-terminus blocked | 9.3–10.0[c] | multiple forms<br>fragment of lactoferrin found in seminal plasma | 82<br>44 |

[a] Values described by Jonáková et al.[33] [b] Theoretical p*I* values obtained from protein sequence database Swiss–Prot. [c] Described by Roberts and Boursnell[82].

– glycoproteins containing N- and O-glycosidically linked oligosaccharide chains,
– simple saccharides or their derivatives.

Various methods were used to prove the saccharide binding site of boar seminal plasma proteins or to study various factors affecting this interaction. Simple saccharides or their derivatives and polysaccharides were used in inhibition studies concerning the inhibition of protein interaction with solubilized zona pellucida[47], of erythroagglutinating activity of isolated proteins[48–50], PSP II binding to glycoproteins containing linked D-mannose-6-*O*-phosphate[51]. Labeled derivatives of glycoproteins, peptides and polysaccharide derivatives were applied in solid phase assays, also called ELBA (enzyme linked binding assay)[52–55]. In the case of polysaccharides, polyacrylamide or ethylenediamine derivatives were used[54]. The same derivatives of ligands were applied for specific detection of electrophoretically separated protein bands blotted to the nitrocellulose membrane. This approach was most often used in the case of detection of zona pellucida binding proteins (e.g. literature[41,56–58]). Affinity chromatography on immobilized ligands was used not only for isolation of proteins with a specific binding activity (e.g. separation of heparin-binding proteins[33,57,59]), but also to prove this binding activity of synthetic peptides or to separate protein domains responsible for ligand binding[60]. In these studies, mostly immobilized heparin[33,57,59] or zona pellucida glycoproteins[61] were used.

TABLE II
Binding properties of boar seminal plasma proteins as determined by solid phase assay (ELBA)[46–48,53,54,77]

| Protein | Phosphoryl choline | Heparin | Chondroitin sulfate | Dextran sulfate | Fucoidan | Hyaluronic acid | Zona pellucida |
|---|---|---|---|---|---|---|---|
| PSP I | – | | – | – | – | – | – |
| PSP II | + | ++ | (+) | (+) | ++ | + | + |
| AWN | +++ | ++++ | ++++ | +++ | +++ | +++ | ++++ |
| AWN 2 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| AQN 1 | + | + | + | + | + | + | ++ |
| AQN 2 | – | ++ | ++ | + | ++ | ++ | ++ |
| AQN 3 | ++ | + | + | + | + | + | ++ |
| DQH | + | +++ | +++ | +++ | +++ | ++ | ++ |

The interaction of isolated boar seminal plasma proteins with acid polysaccharides, zona pellucida glycoproteins and phosphorylcholine studied by ELBA assay and biotinylated polyacrylamide derivatives of the ligands is summarized in Table III[45,46,49,50]. The suggested effect of a different glycosylation degree of PSP proteins[26] on their ability to interact with heparin was not proved in further studies[33].

The interaction of boar seminal plasma proteins with heparin was compared with interactions with other acid polysaccharides of different types: (i) those containing the sulfate groups and differing in their saccharide moiety (chondroitin sulfate, dextran sulfate fucoidan), (ii) that containing only a carboxyl group (hyaluronic acid). The ability of boar proteins to interact with all used acid polysaccharides was very similar[47,49,50]. Bull seminal plasma proteins[62], in contrast to boar proteins, interacted with sulfated polysaccharides significantly more than with hyaluronic acid. Hyaluronic acid was found to participate in capacitation of boar sperm[63] while for capacitation of bull sperm (in vitro), the presence of sulfate groups is necessary[18].

Sperm-coating components of boar sperm are characterized by zona pellucida-binding properties. The ability of isolated spermadhesins to interact with solubilized zona pellucida as determined by ELBA assay is summarized in Table III. The results fully correspond to those obtained by specific detection of blotted spermadhesin zones by biotinylated zona pellucida glycoproteins (e.g. literature[41,56–58,64,65]). O-linked[66] and neutral N-linked saccharide chains from pig ZPB/ZPC mixture were shown to possess a sperm

TABLE III
Aggregated forms of boar seminal plasma proteins and their binding properties[44,46]

| Protein aggregate | Molecular weight | Composition | Heparin binding | ZP binding | Phosphorylcholine binding |
|---|---|---|---|---|---|
| I | >100 000 | DQH, AQN 1-3, AWN 1, PSP IIa, PSP IIb, AWN 2 | +++ | ++ | ++ |
| II | 55 000 | DQH, AQN 1-3, AWN 1-2 | ++ | + | ++ |
| III | 45 000 | DQH, AQN 2-3, AWN 1 | ++ | + | ++ |
| IV | 30 000 | PSP I, PSP IIa, PSP IIb | – | – | + |
| V | 5–25 000 | AQN 1, β-MSP, proteinase inhibitors | – | – | |

ligand activity. Of these complex-type chains, triantennary/tetraantennary chains exhibit stronger activity than diantennary chains[15,67].

Even though boar spermadhesins were shown by different methods to interact with zona pellucida glycoproteins, their role in primary binding is not completely understood. It has been shown that pretreatment of oocytes with the AQN 1 protein resulted in reduced binding of capacitated sperm to oocytes[39,46], or antibodies against the AWN 1 protein interacted with AWN 1 antigens on the plasma membrane of capacitated spermatozoa and inhibited binding of capacitated sperm to the ovum[40]. Based on these findings, the role of these proteins in primary binding was suggested. On the other hand, there exist experiments showing that seminal plasma proteins are released from sperm in the course of capacitation in female reproductive organs and only small amounts of proteins were detected on the sperm surface reaching the ovum[68–71].

Studies on the interaction of isolated boar seminal plasma proteins with different types of glycoproteins were performed to elucidate a possible role of these proteins in the primary binding of sperm to the zona pellucida. Isolated AWN 1 spermadhesin interacted strongly both with N-glycosylated (fetuin) and O-glycosylated (PDC 109, fetuin) glycoproteins containing terminal sialic acid residues[72]. The absence of these terminal acid residues sharply decreased the binding affinity. Spermadhesins AQN 1 and AQN 3 interacted with the same type of glycoproteins as mentioned above, with preference to glycoproteins containing O-glycosidically linked saccharides[42,48]. Contrary to that, the presence of the terminal sialic acid residue significantly decreased the binding activity of AWN 1 spermadhesin[73]. The results obtained by solid phase assays described above using biotinylated glycoproteins[48–50,72,73] were in very good agreement with the results obtained by other methods, such as double diffusion in agarose[42] or by inhibition of the erythroagglutinating activity of boar seminal plasma proteins with different types of glycoconjugates[48–50]. PSP II spermadhesin was found to interact with the oligosaccharide chain of carboxypeptidase containing exposed D-mannose-6-phosphate groups[74].

### *2.3. Interaction of Boar Seminal Plasma Proteins with Components of Sperm Membrane Phospholipids*

Two types of ligands were used to study the interaction of boar sperm membrane phospholipids with boar seminal plasma proteins: phosphorylcholine and phosphorylethanolamine. A specific interaction between non-aggregated AWN1 and AQN3 and phosphorylethanolamine has been re-

ported[75]; it was suggested that these molecules could form the first layer of the coating material by interacting with the membrane lipids.

For the isolation of phosphorylcholine-binding proteins from boar seminal plasma, a similar approach has been used[21] as in the case of the separation of bull phosphorylcholine-binding proteins: proteins interacting with this ligand adsorbed to immobilized heparin were eluted with phosphorylcholine solution[76].

The bulk of boar heparin-binding proteins do not show phosphorylcholine-binding activity. Only AQN1 and DQH proteins were eluted as phosphorylcholine-binding proteins[21,23,33]. Their ability to interact with this ligand was proved by affinity chromatography on immobilized phosphorylcholine derivatives[21,23].

The results of the solid phase assay (ELBA) and using biotinylated L-glycerylphosphorylcholine derivative[77] are shown in Table III: the highest phosphorylcholine activity was found for AWN1 spermadhesin[46].

### *2.4. Interactions Between Boar Seminal Plasma Proteins*

Almost all protein components are present in boar seminal plasma under physiological conditions in the associated forms of different molecular mass, composition and binding properties[25,45,46] (Table II).

Interactions between proteins were investigated by size exclusion chromatography of a mixture of separated monomer proteins and affinity chromatography of boar seminal plasma on immobilized spermadhesins. The composition of proteins that are adsorbed from boar seminal plasma on immobilized spermadhesins corresponds to that of aggregated forms found in seminal plasma. Specific interactions between protein components of boar seminal plasma participate in the formation of aggregated forms of proteins in seminal plasma and most probably also in the arrangement and remodelling of protein coating layers of sperm.

## 3. CONCLUSIONS

In this review we have briefly described the biochemical properties of a group of proteins isolated from boar reproductive tract. Studies on their binding properties point to their possible role in the initial steps of the fertilization process. These steps include binding of seminal plasma proteins to the sperm surface during ejaculation, remodelling of the protein sperm surface coverage, interactions of sperm surface proteins with oviductal epi-

thelial cells, sperm capacitation and primary binding of the sperm to the ovum.

The mechanism of fertilization of the egg with sperm is determined by mutual recognition of both gametes and their binding. Although the interaction mechanism of both gametes has not been fully elucidated yet, many data show that the system of complementary molecules situated on the surface of both gametes plays a key role in this interaction. Damage of the surface proteins of both gametes makes the fertilization impossible or strongly disturbed. Study of the structure of sperm surface as well as of seminal plasma proteins, their characterization and elucidation of principles of their interactions will substantially contribute to the understanding of basic biochemical processes of fertilization. The results of this study will contribute not only to the understanding of molecular interactions involved in various steps of animal reproduction, but may be of practical importance for both veterinary and human medicine, for birth control and in vitro fertilization.

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