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Separation, characterization and identification of boar seminal plasma proteins☆

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Abstract

Methods used for the isolation, separation and characterization of boar seminal plasma proteins are discussed, as well as techniques applied to study their binding properties. Attention is paid to interactions of these proteins with different types of saccharides and glycoconjugates, with membrane phospholipids, and to interactions between proteins. Boar seminal plasma contains different types of proteins: spermadhesins of the AQN and AWN families; 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 of sperm are formed. Sperms coated with proteins participate in different types of interactions occurring in the course of the reproduction process, e.g. formation of the oviductal sperm reservoir, sperm capacitation, oocyte recognition and sperm binding to the oocyte.

Keywords: Boar seminal plasma proteins; Separation methods; Identification and characterization of proteins; Binding properties of proteins; Reproduction; Spermadhesins

1. Introduction

Mammalian reproduction 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 (reviewed in [1–7]).

Seminal plasma proteins participate in events that occur both in the male and the female reproductive tracts. 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 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 reproduction. In the female reproductive tract seminal plasma proteins bound on the sperm surface most probably participate in the formation of the oviductal sperm reservoir, sperm capacitation, oocyte recognition and sperm binding to the ovum [1,6].

The system of complementary molecules situated on the surface of both gametes plays a key role in their recognition and binding. Study of the structure of sperm-coating proteins, their characterization and elucidation of principles of their interactions will contribute to better understanding of the reproduction process. Methods used for separation and characterization of protein components of boar seminal plasma, as well as techniques applied to study their binding properties, are reviewed in this paper.

2. Sample preparation

The study of protein components of seminal plasma with respect to their function in the reproduction process is affected very significantly already in the first step of ejaculate han-

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dling. Unwanted modification of proteins results in the formation of protein artifacts; biological properties of these formed substances may be different from those present in the native state or may be lost. The formation of protein artifacts in the preparative phase may thus negatively affect the whole study. In the phase of sample preparation, it is necessary to avoid a contact of seminal plasma proteins with enzymes released from damaged spermatozoa as well as enzymes present in seminal plasma.

2.1. Separation of spermatozoa and seminal plasma

Sperm plasma membrane should be intact during this preparative step to avoid a release of hydrolytic enzymes to seminal plasma, such as proteases, glycosidases or phosphatases. The presence of some of these enzymes in seminal plasma is considered as a marker of sperm intactness, e.g. hyaluronidase [8].

Conditions of sperm removal from ejaculate ensuring the absence of sperm membrane damage vary from species to species. In pig, the following procedure ensured an intactness of sperm in the course of the preparative step: fresh boar semen was centrifuged at 600 × *g* for 20 min at 15 °C to separate spermatozoa from seminal plasma [9].

2.1.1. Isolation of proteins from boar spermatozoal extract

The spermatozoa were washed twice in sucrose medium pH 6.5 and centrifuged at 1000 × *g* for 20 min at 15 °C. The pellet of spermatozoa was extracted overnight at 4 °C with the same volume of 3% acetic acid, 10% glycerol and 50 mM benzamidine. The acrosomal extract was centrifuged at 14 000 × *g* for 10 min at 4 °C. The clear supernatant containing proteins was purified by a three-step procedure [9–11]. Sperm-free seminal plasma was mixed with 50 mM benzamidine hydrochloride to prevent further proteolytic digestion, and stored at −20 °C.

2.2. The presence of hydrolytic enzymes in seminal plasma

These enzymes change or can change the peptide composition of seminal plasma as well as the pattern of posttranslationally modified proteins. The binding properties of modified proteins (e.g. glycosylated or phosphorylated) might be different from those of unmodified or modified to a lower degree. This situation is complicated by the presence or absence of enzyme inhibitors in seminal plasma. Proteinase inhibitors are present in spermatozoa extracts and seminal plasma. The biological role of acrosin inhibitors is to inactivate the prematurely released acrosin from occasionally damaged spermatozoa and thus to protect the male and female genital tracts against proteolytic degradation. Acrosin inhibitors from boar seminal plasma [12] and sperm-associated acrosin inhibitor (SAAI) are closely related [10,11].

Seminal plasma of different species greatly differs in the content of hydrolytic enzymes. For example, human and canine seminal plasma were characterized by high production of proteases in the prostate, and their high content is related to the modification of protein gel in ejaculate in the first phases of the fertilization process (e.g. [13–15]). In the course of preparation steps for the study of seminal plasma proteins, unwanted action of "endogenous enzymes" should be minimized. In the pig, seminal vesicle fluid contains serine protease activity [16]. For protein isolation, low-molecular-mass protease inhibitors are usually added to seminal plasma to prevent proteolytic digestion.

3. Separation techniques

3.1. Chromatographic methods

Various types of chromatographic methods have been successfully used to separate protein components of seminal plasma of different species. The situation is complicated in boar seminal plasma as well as in other species by the ability of seminal plasma proteins to form aggregated forms [17–20]. These associates differ not only in the size and protein composition, but also in their binding properties. Association of seminal plasma proteins may also change the binding properties of protein monomer forms, as was shown for example in the case of boar spermadhesin PSP I [21]. The nature of associated forms of seminal plasma proteins differs in different species. While almost all proteins of boar seminal plasma are present under physiological conditions in the form of aggregates [17,18,22], the behaviour of bull proteins is different. They are present in seminal plasma in a polydisperse form [23] and substances of the native environment of gametes can modulate their aggregation state [24–26].

The predominating chromatographic separation techniques are affinity chromatography, size exclusion chromatography (SEC), and hydrophobic chromatography (especially reversed phase-high-performance liquid chromatography (RP-HPLC)).

3.1.1. Affinity chromatography

In the separation of boar seminal plasma proteins, the affinity chromatography on immobilized heparin was used as the first step. Proteins were separated by this way into two fractions: heparin-binding (Hep^+) and non-heparin-binding (Hep^-) fractions [27–29]. Both fractions were further separated by RP-HPLC and individual polypeptide chains were isolated. The Hep^+ fraction consisted of DQH sperm surface protein, AQN and AWN spermadhesins, whereas the Hep^- fraction consisted of PSP I and PSP II spermadhesins [27,30]; Hep^+ proteins predominated in aggregated forms II and III (AG II and AG III) with relative mol. mass 55 000 and 45 000, respectively, while Hep^- proteins in the aggregate IV (AG IV) (relative mol. mass 30 000) [18].

A similar approach was used for isolation of protein components of boar prostate secretions [31,32].

In the case of bull seminal plasma proteins, the interpretation of results of affinity chromatography on immobilized heparin is not simple. This type of chromatography was not usually used as the first step of the separation [33–35]. Heparin-binding activity of these proteins depends on their aggregation state, which can be modulated by several factors [20,25,33,36,37].

The elution of adsorbed proteins to affinity carrier-containing immobilized heparin with phosphorylcholine solution was used to separate proteins interacting with this phospholipid [24,27,33,38,39].

Affinity chromatography on immobilized heparin was used not only for protein isolation, but also to prove this type of binding activity of synthetic peptides derived from boar spermadhesins or to separate protein domains responsible for ligand binding [40].

Other types of immobilized ligands were used less frequently. Their application was rather focused on investigation of the binding properties of seminal plasma proteins. For separation of boar seminal plasma proteins, the affinity matrices coupled to the following ligands: L-glyceryl phosphorylcholine [41], gelatin [27], yeast mannan [42,43], spermadhesins [44] and zona pellucida glycoproteins [45] have been described.

Using affinity chromatography with immobilized phosphorylcholine derivative, DQH protein and spermadhesins of AQN family were identified as phosphorylcholine-binding proteins in boar seminal plasma [41]. To study the saccharide-binding activity of boar seminal plasma proteins involved in formation of the oviductal sperm reservoir, yeast mannan immobilized to divinyl sulfone-activated Sepharose was used [43]. The following proteins were identified in the mannan-binding fraction: AQN and AWN spermadhesins and DQH protein.

Affinity chromatography on immobilized gelatin showed a relationship of DQH protein [27,46] from boar seminal plasma to the main proteins of bull seminal plasma [47].

On the other hand affinity chromatography on immobilized spermadhesins (AQN, AWN, PSP) was used to study mutual interactions of protein components of boar seminal plasma. The composition of proteins that are adsorbed from boar seminal plasma to immobilized spermadhesins corresponds to that of aggregated forms found in seminal plasma [44].

3.1.2. Size exclusion chromatography

As was already mentioned above, this type of chromatography was used to show the form of boar seminal plasma proteins in ejaculate under physiological conditions. Almost all protein components are present in seminal plasma in associated forms of different molecular mass and composition [17,18,30]. Besides that, interactions between proteins were investigated by size exclusion chromatography of a mixture of separated monomer proteins [19,44].

3.1.3. High-performance liquid chromatography with reversed phase

RP-HPLC was successfully used for separation and isolation of the monomer forms of proteins, esp. of spermadhesins obtained by affinity chromatography (Hep^+, Hep^- fractions) from boar seminal plasma [27,28,30] or for the analysis of protein fractions after SEC (AG I–AG V) [17–19]. Under suitable conditions, DQH sperm surface protein and individual forms of spermadhesins of AQN family (AQN 1, AQN 2, AQN 3) and AWN family (AWN 1, AWN 2), and PSP I and PSP II were separated [18,27].

3.2. Electromigration methods

Electromigration methods represent an invaluable tool for separation and especially analysis of the protein components of seminal plasma. SDS-polyacrylamide gel electrophoresis, isoelectric focusing, and two-dimensional polyacrylamide gel electrophoresis belong to predominating electromigration techniques used for this purpose. These techniques enable detection of the variability in proteins generated by post-translational modifications. The application of polyacrylamide gel electrophoresis in the study of boar seminal proteins under non-denaturing conditions (native electrophoresis) is limited, probably due to a tendency of low-molecular-mass proteins to form associated complexes as mentioned above (Chapter 3.1.). The combination of isoelectric focusing and SDS-polyacrylamide gel electrophoresis in 2D-gel electrophoresis was used for analysis of the components of human (e.g. [48–51]) or bull [52] seminal plasma.

In the case of boar seminal plasma proteins, SDS-polyacrylamide gel electrophoresis was predominantly used for the characterization of protein composition and molecular mass determination of boar proteins (AQN, AWN, PSP spermadhesins, DQH sperm surface protein, AG I–AG V, proteinase inhibitors) [17–19,30]. In some cases, isoelectric focusing was used, mainly to determine the isoelectric points of spermadhesins and their isoforms (e.g. [27]). A capillary electrophoresis method was developed for separation of boar seminal proteins. Various capillaries and separation conditions were tested; the best resolution was obtained in a bare-silica capillary, with a micellar system consisting of 0.02 M borate buffer pH 10.0 and 0.05 M SDS [53].

As was already mentioned, polyacrylamide gel electrophoresis in the presence of SDS belongs to the most often-used electromigration method in the study of boar seminal plasma proteins. This method yields not only values of rel. mol. masses of protein components and their isoforms, but also the separated protein zones can be further analyzed, e.g. by amino acid sequencing, peptide analysis, matrix-associated laser desorption ionization-time-of-flight-mass-spectrometry (MALDI-TOF-MS), LC-MS/MS (capillary liquid chromatography tandem mass spectrometry).

Detailed proteomic analysis of boar seminal plasma has not yet been published, contrary to other species (e.g. human [54] or bull [55]).

4. Techniques used for identification and characterization of proteins

Protein components of seminal plasma were identified mainly after their separation into monomer forms using different types of separation methods. In the case of identification of boar seminal plasma components, the basic approach involved, besides rel. mol. mass determination (SDS-polyacrylamide gel electrophoresis, MS) was amino acid sequencing (Edman degradation, MALDI-MS, and post-source decay) (e.g. [11,27,28,38,46]).

Combination of different types of MS (e.g. MALDI-TOF-MS) peptide mapping and amino acid sequencing with the effective separation techniques such as 2D-electrophoresis enables identification of minute amounts of proteins. This may prove very valuable especially in the elucidation of the role of

boar seminal plasma proteins in the female reproductive tract (reviewed in [56]).

Based on the results of these techniques, the existence of multiple forms of one polypeptide chain in the boar seminal plasma has been described. These multiple forms are generated by different ways; most probably they result from:

(a) different post-translational modification of the polypeptide chain; e.g. from heterogeneity in the oligosaccharide chain of PSP II spermadhesin [57,58] or from the presence of glycosylated and non-glycosylated forms of a single chain; e.g. AQN 2 is a glycosylated form of AQN 3 [27,59].
(b) the existence of protein molecules with a blocked N-terminal amino acid residue in parallel to the chain with a free amino group; e.g. AWN 2 spermadhesin [27,28].
(c) the occurrence of N-terminally truncated forms [28,43,60].
(d) the existence of "artificially" generated modified peptide chain derivatives due to various factors playing a role in the course of the isolation procedure (discussed in the chapter Sample preparation) that can or should be minimized.

The recognition of multiple forms of boar seminal plasma proteins that can change during the reproduction process is very important as far as the elucidation of the protein function or role is concerned. Such modification can considerably increase the information content and functional repertoire of proteins. Various methods used in proteomics such as 2D-gel electrophoresis, 2D-liquid chromatography, MS, affinity-based analytical methods, are applicable for analysis of these modifications. As far as boar seminal plasma proteins are concerned, more attention has been paid to differently glycosylated isoforms (e.g. [46,59,61]).

Much less information is available on the presence of phosphoproteins in seminal plasma of different species (e.g. boar seminal plasma phosphoprotein was described by Harayama et al. [62]).

Proteins separated by SDS-polyacrylamide gel electrophoresis were, after their transfer to nitrocellulose membrane, specifically detected using labelled ligands of different types. Immunodetection with specific antibodies against individual protein components of boar seminal plasma represents a valuable tool for protein identification, as well as for the search of a relationship between different protein molecules. It may concern individual polypeptide chains of multiple forms in seminal plasma of one species; on the other hand, this approach brings an invaluable tool in the search for homologous proteins in seminal plasma of different species [50,51,63,64].

Immunodetection with specific antibodies against boar spermadhesins was used, e.g. as follows: elucidation of the composition of aggregated forms of boar seminal plasma proteins and mutual interactions of these proteins [19,44] or search for spermadhesins in the boar prostate [31,32].

SDS-electrophoretic separation can also be used to detect the enzyme activity using substrate gel zymography. As this type of electrophoresis is performed under denaturing, non-reducing conditions, so far only hydrolytic enzymes were detected such as proteinase from seminal vesicles [16] or spermatozoa (e.g. proacrosin [9], hyaluronidase [65]).

In the case of boar seminal plasma proteins, the reverse substrate gel electrophoresis was used to show the presence of serine proteinase inhibitors [19].

5. Techniques used to study binding interactions of proteins

Different types of interactions are involved in individual steps of mammalian reproduction. Proteins of seminal plasma that are bound to the sperm surface during ejaculation and that form so-called sperm protein-coating layers, are involved in these events.

Interactions of the lectin type are very important in the entire process of mammalian reproduction. Two of them belong to the most studied binding activities of boar seminal plasma proteins. First, saccharide chains of zona pellucida glycoproteins are supposed to bind receptors present on the sperm surface. The saccharide ligands were first characterized in mice [66] and pigs [67–69]. The ligands of other mammals have not been fully recognized. Recently, Amari et al. [70] described the essential role of the non-reducing terminal α-D-mannosyl residues of the N-linked saccharide chains of bovine zona pellucida glycoproteins in sperm-egg binding.

The second type of protein-saccharide interactions seems to be involved in the formation of the mammalian oviductal sperm reservoir [68,71]; proteins attached to the sperm surface recognize oviductal epithelium glycopeptides. By initiation of capacitation, these associated proteins are shed from the sperm surface. Saccharide specificity of the interaction of sperm surface molecules with epithelial cells appears to vary among species [4,72,73]. In the pig, formation of the oviductal sperm reservoir appears to comprise D-mannose residues [74–76]. In protein components of boar seminal plasma, DQH sperm surface protein and AQN and AWN spermadhesins interacted strongly with porcine oviductal epithelial cells [77].

Little attention has been paid to the investigation of the binding properties of seminal plasma proteins with respect to other types of ligands. Interactions with membrane phospholipids participate in the adsorption of seminal plasma proteins to the sperm membrane during ejaculation. This phenomenon has been thoroughly studied in the case of the bull model [47,78]; less information is available for other species (e.g. stallion [38], boar [41,79]).

Interactions between seminal plasma proteins have not been studied in detail so far. It is now evident that such type of interaction participates in the formation of aggregated forms of seminal plasma proteins and protein coverage of the sperm surface. The existence of aggregated forms of proteins of seminal plasma has been described only for some species [17,18,30,46,80], but very little is known about the physiological role of such association and dissociation processes.

5.1. Techniques to study the saccharide-binding activity of 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 reproduction process. The choice of ligands was directly related to substances present in the natural environment of sperm, both in the male and female reproductive tracts.

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
- neutral polysaccharides
- zona pellucida glycoproteins
- glycoproteins containing *N*- and *O*-glycosidically linked oligosaccharide chains
- simple saccharides or their derivatives

Various methods were used to prove an involvement of the saccharide-binding interactions in individual steps of the reproduction process or to study various factors affecting this interaction. The approaches and methods used for this purpose can be divided into two groups:

(a) direct binding studies:

The interaction of the studied protein with glycoconjugates containing a known saccharide structure, most often with glycoproteins, is investigated first to elucidate the saccharide-binding specifity of the protein (e.g. [18,19,30,32,81–83]). Labelled derivatives (e.g. biotinylated) of glycoproteins and polysaccharides were applied in solid phase assays (ELBA) [18,19,30,32,51,82,83]. Unlabelled polysaccharides were used in a double diffusion method [81,83].

The second example of the use of direct binding studies are experiments with glycoconjugates, obtained from the male and female reproductive tracts (e.g. solubilized zona pellucida, oviductal epithelial cells, polysaccharides, etc.). These studies are performed to find out an endogenous partner in the lectin-like interactions of seminal plasma proteins [18,27,28,30,53,61,84,85]. Biotinylated derivatives of glycoproteins or other ligands were used in solid phase assays (ELBA) [18,27,30] or for the detection of electroblotted proteins after electrophoretic separation [27,28,61,84,85]. In some cases, labelled proteins and unlabelled ligands were applied in the solid phase assay [77].

(b) inhibition studies:

These experiments are performed to find out simple saccharides or their derivatives that inhibit interactions of seminal plasma proteins with complex conjugates mentioned above. The saccharide-binding specificity of the studied protein can thus be better specified. Inhibition studies with simple saccharides may also represent a tool to prove an involvement of saccharide structures in the investigated interactions [43,77].

The following methods are most often used in the study of the saccharide-binding properties of boar seminal plasma:

5.1.1. Detection of electrophoretically separated proteins transferred onto nitrocellulose membrane using biotinylated ligands

Proteins are separated using SDS-polyacrylamide gel electrophoresis and protein zones are blotted onto nitrocellulose membrane. After application of a biotinylated ligand, the complex protein-biotinylated ligand is visualized by means of a product of an enzyme derivative reaction. Horseradish peroxidase coupled to avidin has been used in the majority of cases. This approach was used to show the ability of boar seminal proteins to interact with biotinylated glycoproteins of solubilized zona pellucida [28,61,84,85], or human seminal plasma proteins with biotinylated derivative of heparin [51]. For inhibition studies, an inhibitor can be added to the solution of the biotinylated ligand or can be present, in the case of a rel. high mol. mass substance, in the separation polyacrylamide gel [51].

5.1.2. Solid phase assay also called enzyme-linked binding assay (ELBA)

ELBA tests are analogues to ELISA tests, in which a biotin-labelled derivative of ligand is used instead of labelled antibody. The studied protein was adsorbed to the polystyrene microtiter wells and after application of a labelled ligand, the formed complex protein-labelled ligand is determined spectrophotometrically using a product of the reaction of avidin-peroxidase. For inhibition studies, an inhibitor could be added to the solution of the labelled ligand before incubation with the immobilized protein.

This method has been successfully used to determine the binding properties of HPLC-separated protein components of boar seminal plasma, their associated forms isolated using SEC [17–19,30] and to show the ability of seminal plasma proteins to interact with components of the female reproductive tract [43,77].

5.1.3. Affinity chromatography

This type of chromatography may be used not only to isolate but also to prove or show the specific binding activity of separated proteins, synthetic peptides or protein domains responsible for ligand binding. In the case of boar proteins, immobilized heparin [40] or zona pellucida glycoproteins [45] were used. Affinity liquid chromatography on heparin immobilized to Toyopearl was used to study the interaction of boar, bull and human seminal plasma with heparin and phosphorylcholine. Relative amounts of heparin-binding and phosphorylcholine-binding proteins in seminal plasma of three species were determined [53].

5.1.4. Double diffusion in agarose gel

This method is not very often used at present in connection with saccharide-binding activities of boar seminal plasma proteins. However, it yields reliable results that are in very good agreement with those of other methods [81].

For detection of protein zones on nitrocellulose membrane and for the solid phase assay, preparation of biotin-labelled derivatives is necessary. Proteins or glycoproteins (e.g. spermadhesins) and peptides are biotinylated directly using a suitable biotin derivative. In the case of polysaccharides containing free

carboxyl groups, the carbodiimide reaction was used to couple them either to ethylenediamine or to poly(acrylamide-allyl amine) copolymer [86,87]. Neutral polysaccharides were first periodate-oxidized and then coupled to the same substances as mentioned above for the carbodiimide reaction [86]. Free amino groups of polyacrylamide or ethylenediamine derivatives of both the neutral and the acidic ones were used for the labelling.

A number of binding studies with boar seminal plasma proteins were performed with proteins separated by RP-HPLC and/or by SDS-polyacrylamide gel electrophoresis (electrophoresis under denaturing conditions). In evaluating the results, it is necessary to take into consideration the nature of the separation methods. Besides that, the results concern the binding activity of monomer forms of proteins that are not to be present in seminal plasma under physiological conditions. It has been shown that the binding properties of associated forms of boar spermadhesins not always correspond to those of their components [7,18].

5.2. Techniques to study the interaction of boar seminal plasma proteins with components of sperm membrane phospholipids

The amount of information concerning the interaction of seminal plasma proteins with components of membrane phospholipids is limited along with the methods used for their study. The following techniques have been used:

5.2.1. Affinity chromatography on immobilized heparin followed by elution of the adsorbed proteins with phosphorylcholine solution

This approach was used to isolate phosphorylcholine-binding proteins from bull seminal plasma [37] and to show the presence of such proteins and compare their amounts in seminal plasma of other species [38,41,53].

5.2.2. Affinity chromatography on immobilized phosphorylcholine derivative

A component of sperm membrane phospholipids was coupled as *p*-aminophenyl phosphorylcholine to Agarose [88] or as L-glyceryl phosphorylcholine to divinylsulfone-activated Sepharose [41]. The content of phosphorylcholine-binding proteins in seminal plasma of different species was compared.

5.2.3. Solid phase assay also called ELBA

An analogous biotin-labelled ligand derivative was prepared as in the case of the study of saccharide-binding properties. An L-glyceryl phosphorylcholine derivative coupled to poly(acrylamide and allyl amine) copolymer [89] after biotinylation was used for direct binding of this ligand to separated protein components of boar seminal plasma and to their aggregated forms [18].

5.3. Techniques used to study the 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 [17,18,30], and could be separated using size exclusion chromatography.

The following methods were used to show that the formation of aggregated complexes is not accidental but that specific interactions exist between proteins that participate in this process.

5.3.1. Affinity chromatography on immobilized monomeric forms of spermadhesins

Complete boar seminal plasma proteins or their different fractions were applied to an affinity carrier and the retained proteins were analyzed. The composition of proteins adsorbed to immobilized spermadhesins corresponded to that of aggregated forms found in seminal plasma [44].

5.3.2. Size exclusion chromatography

Size exclusion chromatography was used to show the ability of monomer forms of boar seminal plasma proteins to associate specifically in higher molecular mass aggregates. Different mixtures of monomer proteins were incubated under physiological conditions and separated based on their rel. mol. masses [19,44].

6. Conclusions

In this review we have briefly described the methods that were used to isolate and separate a group of proteins from the boar reproductive tract and the techniques applied for their identification and characterization. Attention was further been paid to the methodological approaches used to study the binding properties of these proteins.

Studies on the binding properties point to the possible role of these proteins in the initial steps of the reproduction 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 epithelial cells, sperm capacitation, and primary binding of the sperm to the ovum.

The mechanism of fertilization of the egg by 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 to 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 reproduction.

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