

Characteristics of selected seminal plasma proteins and their application in the improvement of the reproductive processes in mammals

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Abstract

Understanding the biochemical processes associated with ovum fertilization and knowledge about the structure and function of individual substances participating in these processes is crucial for the development of biotechnological methods to improve reproduction of animals and humans. Among many components of seminal plasma, proteins and peptides play a specific role in regulation of the fertilization process, particularly through their ability to bind various types of ligands such as polysaccharides, lipids and ions. Heparin-binding proteins regulate capacitation and acrosome reaction processes. Affinity of plasma proteins to mannans of the fallopian tube epithelium facilitates formation of spermatozoa reservoirs in the female reproductive tract. Ability to bind phosphocholine is one of the conditions for the coating of the seminal plasma proteins on the sperm membrane and also determines the formation of oligomeric forms of certain proteins. Zinc binding by seminal plasma proteins regulates sperm chromatin condensation state. It also affects motility of these cells and acrosome reaction. The interspecies analysis indicates significant structural and functional similarities, especially for the proteins with low molecular weight. Fertility associated proteins (FAPs) have been determined in the bull, stallion, boar, ram and dog. The contents of these proteins correlate with the indicators of the fertilizing abilities of sperm. In humans, several seminal plasma proteins were found which serve as diagnostic markers of spermatogenesis, seminiferous epithelium state, and azoospermia. To determine the semen ability for preservation, measurement of some seminal plasma protein content may also be used. Addition of specific plasma proteins to a spermatozoa solution undergoing the process of preservation may be used to retain the features of the cells responsible for efficient fertilization.

Key words: seminal plasma, proteins, polypeptides, fertility-associated proteins (FAPs)

Introduction

To fully understand the molecular mechanisms associated with ovum fertilization, and then use that knowledge to improve the technological processes, it is necessary to learn, apart from the structure, the interactions between proteins and ligands and their resulting functions. Here, a new branch of proteomics appears, which is interactomics i.e., proteomics of interaction. It combines study of both the interactions, and the consequences of these interactions for functions of proteins and other cell molecules (Park et al. 2005).

Modern research methods, such as chromatography, flow cytometry, one-dimensional gel electrophoresis (SDS PAGE), two-dimensional gel electrophoresis (2 D PAGE), and mass spectrometry (MS), enable the characterization of proteins. Knowledge about immunological properties, enzymatic activities, susceptibility to proteolysis, ability to bind various ligands, and protein-protein interactions, results to interactomics, which facilitates understanding of the impact of proteins on selected indicators of semen quality.

Recent research focused on the possibility of application of the knowledge about structure and function of seminal plasma proteins in the improvement of semen storage in liquid or frozen state, and on searching for individual proteins, which could have served as markers of male fertility or diagnostic markers for diseases of accessory sex glands of the reproductive tracts.

Functional characteristics of ligand-binding seminal plasma proteins

Mammalian seminal plasma is the liquid part of ejaculate, which is composed of secretions of testes, epididymides, vasa deferentia and fluids of male accessory sex glands. Among the wide range of components of the seminal plasma, the peptide and protein substances have a specific role in the regulation of fertilization process. Most of the proteins found in the seminal plasma bind to plasma membrane of spermatozoon and coats its surface, preventing sperm agglutination, premature acrosome reaction and phagocytosis in the female reproductive tract (Jonakova and Ticha 2003).

The fertilization process includes a number of specific and tightly coordinated reactions. Association of seminal plasma proteins with the surface of sperm occurs during ejaculation. Subsequently, interaction of the substances with components of the epithelial cells of the fallopian tube, capacitation, specific gametes recognition, primary and secondary binding of sperm to ovum, acrosome reaction, sperm penetration

through zona pellucida, and finally fusion of the gametes occur in the female reproductive tract. All the reactions are characterized by precise regulation mechanisms based on protein-ligand interactions (Manaskova et al. 2003).

Seminal plasma proteins demonstrate specificity against different ligands. Apart from the interaction with polysaccharides, phospholipids, lipoproteins, bivalent ions, seminal plasma proteins recognize receptors of sperm plasmalemma and zona pellucida. It was recently discovered that seminal plasma proteins could also form protein-protein complexes, which develop to aggregated forms (Manaskova et al. 2000).

Saccharide-based interactions of seminal plasma proteins

Heparin- or chondroitin-sulfate-like glycosaminoglycans (GAGs) are secreted in large amounts by the female reproductive tract epithelium, particularly during follicular phase of the estrous cycle (Calvete et al. 1996). Changes in GAGs content on sperm plasma membranes occur during sperm transport in the female reproductive tract. Dissociation of those substances from the surface of plasma membrane during sperm capacitation presumably starts a heparin-dependent process of Ca^{2+} ions increase in acrosome matrix. It was found that heparin-binding seminal plasma proteins also contribute to the acrosome reaction by interacting with heparin-like GAGs of the fallopian tube after attachment to the sperm surface (Florman and First 1988).

Recently, it was discovered that the seminal plasma proteins, which bind mannose, participate in formation of sperm reservoirs in the fallopian tube. The phenomenon of sperm storage in the oviduct isthmus is associated with the acquisition of the fertilizing ability by the above-mentioned cells. The released spermatozoa undergo hyperactivation and they have increased calcium concentration in acrosome matrix (Green et al. 2001). The proteins present on the surface of the sperm plasma membrane recognize mannose glycoproteins, which occur in the epithelium of the fallopian tube. Oviductal sperm reservoirs are formed and exist until the moment of capacitation, when the proteins associated with plasmalemma are removed from it (Jelinkova et al. 2004a).

Phospholipid-based interactions of seminal plasma proteins

Sperm plasma membrane coating with proteins secreted by accessory sex glands during ejaculation is

completed among others due to the interaction with choline phospholipids of the plasma membranes (Gasset et al. 1997). Phospholipids, which contain phosphorylcholine, constitute more than 60% of all phospholipids in the sperm membranes. Phosphorylcholine is a component of phosphatidylcholine and together with phosphatidylethanolamine constitute the main phospholipid components of sperm membranes (Parks et al. 1987).

Proteins that bind phosphatidylcholine have been identified in many species of mammals like cattle, pig, hamster, rat, mouse and human (Leblond et al. 1993). In case of stallion and boar, these substances exhibit capability of binding both phosphorylcholine, as well as heparin. It was stated that structurally, they are similar to bovine seminal plasma proteins (BSP), and show ability to form oligomeric structures that in the presence of phosphorylcholine disintegrate into homodimers (Calvete et al. 1997).

Aggregation and disaggregation of seminal plasma proteins is probably an important phenomenon during fertilization process. In case of seminal plasma of boar, interactions between spermadhesins families AQN and AWN, and DQH, as well as between the proteins participating in PSP-I and PSP-II heterodimer formation were demonstrated. Plasma proteins showing affinity to phosphorylcholine can play an important role in the discussed interactions (Manaskova et al. 2000).

Zinc-based interactions of seminal plasma proteins

Seminal plasma proteins of mammals demonstrate high affinity to zinc ions. These ions may be temporarily or permanently bound structural component of seminal plasma proteins. Therefore, they may participate in the formation of the final structure of proteins or be used as a mobile regulation element of many processes accompanied to fertilization.

Zinc ions are involved in the intracellular mechanism protecting the stability of sperm chromatin. They participate in the formation of S-Zn-S type bonds in protamine structure, which additionally stabilizes chromatin. Thus, maintaining an adequate level of zinc ions in the sperm chromatin determines its function in the fertilization process (Bjorndahl and Kvist 2010).

Zinc ions bound to seminal plasma proteins play a specific role in the discussed mechanism of sperm chromatin stabilization (Kvist 1980). Dysfunctions of the male accessory sex glands, which are the source of seminal plasma proteins, negatively affect the stability of chromatin, because the normal concentration of

protein, which binds zinc ions, regulates their content in sperm chromatin (Kjellberg 1993).

Zinc ions are removed from sperm during epididymal transportation, which causes disulphide bridges formation in proteins and development of a structure, which enables better energy use. In the bull, zinc-binding proteins were observed in epididymal fluids (Henkel et al. 2001).

Protein called seminal vesicle autoantigen (SVA) was described in mouse males. This protein has the ability to form complexes with zinc ions and bind choline phospholipids of sperm plasmalemma. SVA acts as a decapacitation factor (Huang et al. 2000).

Zinc-dependent proteins, which are present in the semen, can be classified as substances protecting the supply of zinc ions by their binding and transport. Disturbances of synthesis of these proteins or their typical biological activity can lead to physiological dysfunctions of spermatozoas.

Species-specific ligand-binding seminal plasma proteins

Seminal plasma proteins were isolated and characterized based on their ability to bind ligands. Proteins demonstrating the affinity to heparin have been most extensively investigated and described, while there is lack of publications about other ligands. However, it was suggested that the same protein may possess the ability to bind several various ligands at the same time (Calvete et al. 1997, Manaskova et al. 2000). In this study, there is only brief description of the best-known seminal plasma proteins in individual species.

Human

Kumar et al. (2009), using two-dimensional (2-D) electrophoresis and mass spectrometry (MS), performed proteomic analysis of human seminal plasma. Authors identified approximately 70 proteins showing an affinity to heparin. Analysis of functionality showed that 38% of these proteins exhibit enzymatic activity, 20% participate in RNA processing and transcription, 18% performs structural and transport functions, 16% are proteins recognizing and transducing signals and 8% is of unknown function. Heparin-binding proteins take part in capacitation and acrosome reaction. Semenogelin I, semenogelin II, fibronectin, lactoferrin, and prostate specific antigen (PSA) are part of this group. PSA is used as a marker of prostate cancer (Gobello et al. 2002).

Best-known zinc-binding proteins of human seminal plasma are semenogelins (Robert and Gagnon

1999). They participate in coagulum formation, regulate DNA stability, inhibit sperm movement (Robert and Gagnon 1999, de Lamirande 2007, Yoshida et al. 2008), and possess antibacterial activity (Edstrom et al. 2008). In addition, they hyperpolarize sperm plasma membranes (Yoshida et al. 2008) and prevent capacitation (de Lamirande et al. 2001).

Zinc is secreted by human prostate gland in two forms: free and associated with high molecular weight protein complexes. Prostatosomes are small lipoprotein vesicles containing zinc, which is secreted by the prostate to the human seminal plasma and acts as a regulator of physiological properties of sperm. Beside chromatin stabilization, prostatosomes participate in acrosome reaction and sperm motility regulation. They exhibit immunosuppressive, antioxidative, and antibacterial properties (Vivacqua et al. 2004).

Boar

Low molecular weight spermadhesins prevail in boar seminal plasma. These are adhesive proteins, which coat the boar sperm after ejaculation. Six spermadhesins have been characterized so far: AQN-1, AQN-2 (PSP-I), AQN-3, PSP-II, AWN-1, AWN-2 (Calvete et al. 1994b). Spermadhesins are multifunctional proteins, which besides the ability to bind heparin (AQN-1, AQN-2, AQN-3, and AWN) and serine protease inhibitors (AQN-1, AWN) demonstrate activity similar to lectins. Post-translational modifications, such as glycosylation, condition the diversity of functional properties of boar spermadhesins (Calvete et al. 1994c).

Kwok et al. (1993b) isolated PSP-I and PSP-II proteins from boar seminal vesicles secretions. The substances form heterodimer PSP-I/PSP-II (Calvete et al. 1997). PSP-I/PSP-II heterodimer does not bind heparin, but shows affinity to glycoproteins of the zona pellucida and trypsin inhibitors. Spermadhesin PSP-I binds, among others, endo- β -galactosidase, which digests ZP3 and α -casein, and shows the ability to neutralize trypsin inhibitor from soybean, which may indicate the participation of this protein in counteracting pre-mature acrosome reaction (Kwok et al. 1993b). Moreover, PSP-I exhibits the ability to bind IgG of various animal species and human IgA (Kwok et al. 1993a). Thus, it can function as immunosuppressive factor to protect sperm from an immune reaction during its transport in the female reproductive tract. PSP-I/PSP-II heterodimer is also called immunosuppressive factor of boar seminal plasma (ISF – immunosuppressive seminal factor) (Veselsky et al. 2002).

A 54 kDa glycoprotein (Gp 54), isolated from boar seminal plasma and seminal vesicles, belongs to

proteins that are capable of forming multifactor aggregated forms. It contains three spermadhesins (AQN-3, AWN, and pB1 (DQH)). The glycoprotein shows the ability to precipitate lipoproteins, hemagglutinating and anti-trypsin activity. In addition, it inhibits spontaneous and induced lymphocyte proliferation (Strzezek and Holody 1996, Plucienniczak et al. 1999). Peptide sperm motility inhibiting factor (SMIF) was found in composition of pB1 subunit (5.7 kDa) (Kordan et al. 1998). SMIF inhibits sperm motility through rapid lowering of ATP level in the discussed cells. This peptide also demonstrates antibacterial properties.

Recently, platelet-activating factor acetylhydrolase (PAF-AH) was isolated and purified from boar sperm. The protein is composed of four polypeptides of 43, 55, 65, and 100 kDa. Alpha-mannosidase, fibronectin, AWN-1 spermadhesin, PSP-II, and IgG-binding protein were identified in subunit composition (Kordan et al. 2007). The discussed enzyme hydrolyzes platelet-activating factor (PAF), a glycerophospholipid occurring in the sperm plasmalemma. PAF enhances sperm motility, capacitation, and acrosome reaction (Kordan et al. 2010). The amount of PAF released from sperm is regulated by the activity of PAF-AH, which indicates its important function in the regulation of sperm activity (Kordan et al. 2003).

Using concanavalin A affinity chromatography, it was demonstrated that poly-mannose glycoproteins occur in boar sperm plasma in large amount, especially in the form of low molecular spermadhesins (Strzezek et al. 2002). Boar seminal plasma proteins (AQN 1, AWN, and DQH, and their aggregated forms), which bind heparin, show affinity to mannans. Moreover, they possess the ability to bind to the epithelium of the fallopian tube (Jelinkova et al. 2004a,b).

A zinc-binding protein secreted by boar seminal vesicles has been isolated from the seminal plasma. It possesses antibacterial properties and regulates sperm motility (Strzezek et al. 1987b, Strzezek and Hopfer 1987a). Recent study revealed that zinc-binding proteins of boar seminal plasma form high molecular weight aggregates in their native state. Under denaturing and reducing conditions (2-D PAGE) they showed 148 polypeptides with isoelectric points mostly in the basic and neutral pH range. Low molecular weight of these substances indicates their similarity to the spermadhesins family (Mogielnicka-Brzozowska et al. 2011).

Bull

Bull heparin-binding seminal plasma proteins, which bind heparin, have been isolated both from

ejaculated and epididymal spermatozoas. They were characterized by wide range of molecular weights from 15-17 kDa to 30 kDa. All of them were included to main bull seminal plasma proteins – BSP, among which the following fractions: BSP-A1, BSP-A2, BSP – 30 kDa can be distinguished (Manjunath and Therien 2001). BSP-A1 and BSP-A2 are two differently glycosylated forms of the same protein, which constitute individual chemical units. Both the proteins are also called PDC-109 (Esch et al. 1983).

It is suggested that glycosylation process can regulate receptor functions of spermadhesin isoforms. Activation of receptor capability between capacitation factor (heparin binding) and zona pellucida-binding protein can be as an example (Calvete et al. 1994c).

Mannose-binding PDC-109 protein, which is synthesized in seminal vesicle gland, participates in formation of sperm reservoirs in the female fallopian tube. Attaching sperm to epithelium of the fallopian tube through the discussed protein prolongs its life until capacitation. PDC-109 proteins play a significant role in modifying sperm lipids during capacitation and acrosome reaction (Calvete et al. 1999, Suarez 2006).

Clusterin can be identified among heparin-binding proteins localized both in seminal plasma and on sperm cells (Howes et al. 1998). It is a glycoprotein with two heparin-binding sites in its structure. Its role in lipid transport is attributed to it.

Stallion

Stallion seminal plasma proteins, which bind heparin, have been isolated from ejaculated sperm. HSP-1 and HSP-2 are included in the same family as bull seminal plasma proteins – BSP-(A1/A2) and -A3. It was observed that they constitute over 70% of all proteins that bind heparin. HSP-I is a component, which constitute about 12% of all proteins, which bind heparin and coat the sperm head. This component is not glycosylated, and it is immunologically similar to boar spermadhesin AWN (Calvete et al. 1994a, Calvete et al. 1995). HSP- 3 has been shown to be a member of the cysteine-rich secretory protein (CRISP) family (Schambony et al. 1998). HSP- 4 is related to a calcitonin gene-like product. Calcitonin levels have been shown to correlate with sperm motility (Mungan et al. 2001). HSP- 5 can not be related to known proteins. HSP- 7 is the homolog of boar spermadhesin AWN (Reinert et al. 1996). HSP 6 and 8 belong to the kallikrein-like protein family. These proteins demonstrated homology with human

PSA (Topfer-Petersen et al. 2005, Jonsson et al. 2005).

Dog

SDS-PAGE electrophoresis has demonstrated 37 protein fractions of 3 to 100 kDa in dog seminal plasma. Nineteen of them bind heparin (5.2 – 61.5 kDa). Functions of heparin-binding proteins in dog seminal plasma have not been fully recognized yet. They probably take part in modulation of acrosome reaction, and the concentration of some of them correlate with the male fertility (FAPs – fertility associated proteins) (de Souza et al. 2006).

Some zinc-binding proteins have been characterized in the dog (Johnson et al. 1969). A considerable portion of the zinc in the prostatic fluid appears to be bound to these proteins.

Proteins considered as “fertility markers”

Attaching the seminal plasma proteins on sperm cells is connected with stabilization of plasmalemma components, masking antigens exposed on the surface of these cells, prevention of pre-mature acrosome reaction and protection of sperm membranes from harmful influence of lipids peroxidation. The above-listed functions are the basis for searching the biological markers in seminal plasma, which might serve as diagnostic indicators of fertilization capability of sperm (Strzezek et al. 2005).

Currently, there is a tendency in the market to introduce commercial diagnostic kits, which enable easy and quick determination if a given male should be qualified to high quality breeders. It is connected with use of his semen for insemination in the future and obtaining offspring with desired characteristics. Such tests are used in cattle, and are based on detection of protein considered as fertility marker – fertility associated antigen (FAA) (Bellin et al. 1998, McCauley et al. 1999).

Using 2-D PAGE, Kilian et al. (1993), identified four bull seminal plasma proteins, which were connected with bull fertility. Two of them prevailed in bulls with increased fertility (26 kDa, pI 6.2 and 55 kDa, pI 4.5). They were characterized as prostaglandin D synthase and osteopontin (OPN) (Cancel et al. 1997, Gerena et al. 1998). Extensive research on 1000 bulls proved that OPN content in seminal plasma is highly positively correlated with the fertility of these animals (Amman and Hammerstedt 2002; Moura 2005). This protein was also identified in stallion – SP-1 of 72 kDa, pI 5.6 (Brandon et al. 1999). In dog seminal plasma, three isoforms of os-

teopontin of 46.4, 37.7, and 36.5 kDa were recognized (de Souza et al. 2009). It is a multifunctional protein, present in many tissues and secretions of an organism (among others in seminal plasma and accessory glands). It participates in cell adhesion, tissue reconstruction, immune system cell stimulation, chemotaxis, intracellular signal transduction (Moura et al. 2010).

In contrast to proteins, which positively influence the parameters of male fertility, proteins occurring in increased amounts in males with diminished fertility are also known. In bulls, these proteins have 16 kDa, pI 4.1 and 16 kDa, pI 6.7 (Kilian et al. 1993). In stallion three of these type proteins were identified: SP-2 (75 kDa, pI 6.0), SP-3 (18 kDa, pI 4.3), and SP-4 (16 kDa, pI 6.5) (Brandon et al. 1999). Novak et al. (2010b) confirmed negative influence of some seminal plasma proteins on stallion male fertility: SP1 (14kDa), SP2 (19.2 kDa), kallikrein (1E2) and clustrin. But also found that cysteine-rich secretory protein 3 (CRISP3) is a good positive marker of stallion fertility.

In boar seminal plasma, four FAPs were determined. Two of them (55 kDa, pI 4.8 and 26 kDa, pI 6.2) showed positive correlation with fertility. High concentration of the discussed proteins in boar seminal plasma was positively correlated with the fertilization capability of semen, both under *in vitro*, as well as under *in vivo* conditions. The phenomenon was manifested by a higher percentage of fertilization and number of piglets born alive per litter (Flowers 2000, Flowers 2009).

The latest research of Novak et al. (2010a) showed that the number of piglets born alive and the motility of sperm on the seventh day of storage were negatively correlated with the concentration of a 22 kDa protein, identified with use of mass spectrometry as PSP-I. Index of fertility and farrowing rate were positively correlated with a 25 kDa protein, defined as glutathione peroxidase (GPX5). It is an antioxidative enzyme, which protects sperm membranes from oxidative stress damage.

The following proteins: 03 (7.9 kDa, pI 6.35); 23 (13.6 kDa, pI 5.01), and 31 (21.4 kDa, pI 4.75) are components of ram seminal plasma, which may potentially be considered as fertility markers (FAPs) (Moura et al. 2010).

De Souza et al. (2007) identified two proteins considered as fertility markers in seminal plasma of dog. Molecular weights of these molecules were 67.0 and 58.6 kDa. It was stated that the content of these proteins is positively correlated with the motility of sperm, viability, percentage of morphologically normal cells, and integrity of plasma membranes.

Several seminal plasma proteins, which serve as

diagnostic markers of spermatogenesis, condition of the seminiferous epithelium, or azoospermia were identified in humans.

Lactose dehydrogenase C4 (LDH-C4), which occur in seminal plasma, originates mainly from damaged or destroyed cells and can be a marker of seminiferous epithelium condition (Virji and Naz 1995).

Low level of anti-Mullerian hormone (AMH) was determined in seminal plasma of men with changed sperm motility (Fallat et al. 1996) or with spermatogenesis disorder (Fenichel et al. 1999).

Starita-Geribaldi et al. (2001), using 2-D electrophoresis, showed the lack of three groups and seven individual polypeptides of pI ranging from 4.6 to 6.2 and molecular weight of 41-18 kDa on the polypeptide map of seminal plasma of individuals submitted to vasectomy.

Lipocalin-type prostaglandin D synthase (L-PGDS) can serve as a marker for azoospermia in humans (Heshmat et al. 2008). It is a glycoprotein of 26 kDa. Functions of this enzyme in the male reproductive system have not been fully clarified yet; however, it is considered that it participates in sperm maturation in the epididymis (Leone et al. 2002).

As it was mentioned before, prostate is a source of a number of substances participating in fertilization process. Therefore, violation of the physiological state of the gland can disturb the normal reproductive processes. Protein, which might be a unique marker for prostate cancer is still under investigation. The first protein considered as such marker in humans was prostate acidic phosphatase (AcP) (Taira et al. 2007). At present, research on AcP activity is believed to be of lesser importance, while PSA is assayed more frequently (Gobello et al. 2002). PSA is a glycoprotein produced by epithelial cells of the prostate, secreted to semen. It belongs to serine proteinases of kallikrein group (Bell 1995). PSA shows high homology to the main protein of dog prostate secretions – canine secretory prostatic esterase (CPSE) (Isaacs and Coffey 1984). Both enzymes belong to serine proteinase group and show similar activity in relation to protein substrates. Although CPSE is a trypsin-like enzyme, and PSA shows chymotrypsin activity, they both possess similar molecular weight, 29 kDa and 34 kDa, respectively (Dube et al. 1986, Clements 1989).

The possibility of technological use of seminal plasma proteins

Semen preservation and its long-term storage are important for livestock farmers, scientists, and physicians. However, stages of technological proceedings,

both during semen liquid preservation, and cryopreservation, may impair the stability and functions of sperm ultrastructures, mainly the plasmalemma. This results in decreased fertilization capability of sperm. Especially, the high dilution of sperm causes the removal of part of seminal plasma proteins adsorbed after ejaculation, including natural antioxidants and ions crucial for keeping the integrity and functions of plasmatic membranes. This results in the deterioration of semen quality and reduces the process of fertilization. Addition of small amount of seminal plasma or serum albumin to the solvent, in which sperm is stored, allows neutralization of some undesired effects (Maxwell and Johnson 1999).

Studies concerning use of spermadhesin heterodimer PSP-I/PSP-II of boar seminal plasma as additive to the solvent are very interesting. Incubation of boar sperm with isolated fraction of PSP-I/PSP-II or PSP-II spermadhesin positively influenced the preservation of their biological properties, especially high potential of mitochondrial membranes and sperm motility. The discussed effects were weaker when sperm was incubated solely with PSP-I spermadhesin (Centurion et al. 2003).

PSP-I/PSP-II, PSP-I, and PSP-II submitted to trypsin degradation caused effects similar to those caused by their native forms. Five-hour incubation of sperm with peptide fraction of PSP-I/PSP-II devoid of oligosaccharide moieties affected in higher percentage of sperm with high fertilization capability in comparison to the sperm incubated with native dimer solution or with sugar fraction (Garcia et al. 2006). It turned out that PSP-I and PSP-II subunits exert different influence on the biological properties and the protective effect was caused mainly by the peptide part of these proteins.

Seminal plasma proteins as markers for sperm suitability for cryopreservation

The technology of cryopreservation of sperm induces changes in sperm structures similar to capacitation and acrosome reaction (Kumar et al. 2003). Cold shock and osmotic shock associated with cryopreservation of sperm can result to changes in lipid composition of plasmalemma, thus influencing its fluidity. This phenomenon induces increase in membrane permeability, which results in enzyme leakage and changes in membrane channel permeability. In case of boar sperm, the type of fatty acids and differences in the composition of phospholipids in comparison with the sperm of other species, are the cause of high susceptibility to cold shock (de Leeuw et al. 1990).

Seminal plasma of boars qualified as so called "good freezers", positively influences the functions of

sperm submitted to cryopreservation, because it increases the resistance to cold shock (Hernandez et al. 2007).

Our laboratory results indicate that zinc-binding proteins of boar seminal plasma have a shielding effect on the plasma membrane and acrosome of spermatozoa, protecting these structures against consequences of cold shock (Mogielnicka-Brzozowska et al. 2011).

Jobim et al. (2004) observed differences in protein profiles of seminal plasma of bulls with ejaculates of high or low suitability for freezing. Analysis of low molecular weight proteins (10-30 kDa) of bull seminal plasma, using 2-D electrophoresis, showed the presence of four proteins (15-16 kDa, pI 4.7-5.2, 11-12 kDa, pI 4.8-4.9, 13-14 kDa, pI 4.0-4.5, 20-22 kDa, pI 4.8-5.2) in bulls ejaculates of increased suitability for freezing. On the other hand, a protein of 25-26 kDa, pI 6.0-6.5, occurred at higher concentration in ejaculates of decreased suitability for freezing. A protein of 11-12 kDa was identified as clusterin (20-22 kDa) described earlier (Jenne et al. 1991). During freezing of bull sperm, loss of sperm surface proteins such as BSP A1/A2, BSP-A3, and BSA-30 kDa, occurs from 40-57% to 4-6%, which is connected with the increase of pre-maturely capacitated cell percentage (Nauc and Manjunath 2000).

Barrios et al. (2005) stated that goat seminal plasma could have protective functions for sperm plasma membranes towards cold shock. Two seminal plasma proteins were responsible for the discussed protective effect: one 14 kDa and another one 20 kDa. Amino acid sequence of the 14 kDa protein exhibits high homology with several seminal plasma spermadhesins of other species, which possess fibronectin type II domain. The 20 kDa protein did not show any homology with any of known seminal plasma proteins.

The effect of dialysis on semen quality following cryopreservation is very interesting. It was shown that during dialysis, changes in the structure or in content of individual protein components might occur. Such dialyze-based effects had a positive impact on the sperm quality. These changes were manifested in increased post-thaw sperm motility, viability, and mitochondrial function. Furthermore, semen dialysis prior to freezing suppressed the peroxidation of sperm plasma membranes lipids (Fraser et al. 2007).

The findings of these studies show that there are technological problems regarding the development of more efficient methods for semen cryopreservation.

Conclusions

Mammalian seminal plasma is a carrier for sperm and contains a number of factors crucial for normal

fertilization. Polypeptide and protein function as regulators at many stages of the process discussed, due to the ability to bind different oligosaccharide, lipid, and ionic ligands. Affinity for heparin was found to be a primary feature of seminal plasma proteins in most species of mammals. Proteins that bind polysaccharide regulate capacitation and acrosome reaction processes. Affinity of plasma proteins to mannans of the fallopian tube epithelium enables the formation of sperm reservoirs in the female reproductive tract. The ability to bind with phosphorylcholine is one of the conditions needed to enable coating of the sperm membrane with seminal plasma proteins, and decides about formation of oligomeric forms of some proteins. Zinc ions binding by seminal plasma proteins regulate sperm chromatin condensation state, motility of these cells, and acrosome reaction. Interspecies analysis indicates considerable structural and functional similarities, especially among seminal plasma spermadhesins.

The content of some seminal plasma proteins demonstrates correlation with the indicators of sperm fertilization capability. Fertility associated proteins (FAPs) were characterized in the bull, stallion, boar, ram, and dog. In humans, several plasma proteins were characterized, which serve as diagnostic markers of spermatogenesis, seminiferous epithelium condition, and azoospermia. Proteins, which could be unambiguous marker for prostate cancer, are still sought.

Measurement of some seminal plasma protein content can also be used in order to state the suitability of sperm for preservation. Addition of specific plasma proteins to sperm mixture to retain their features responsible for efficient fertilization after storage is also possible.

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