REPRODUCTIVE BIOLOGY

Secretory activity of boar seminal vesicle glands

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Received: 2 October 2002; accepted: 30 October 2002

SUMMARY

This paper reviews the recent research on the physiological role of peptide and protein substances synthesized in boar seminal vesicle glands. Secretions from these glands are the major components of seminal plasma. Unique biochemical properties of seminal vesicle proteins, which determine their numerous functions in the reproductive processes, are discussed. The discovery of phosphotyrosine acid phosphatase in boar seminal vesicle secretions and the first description of the biochemical properties of platelet activating factor acetylhydrolase (PAF-AH) have led to remarkable inroad in molecular andrology. The fundamental role of vesicular low and high molecular antioxidants in the protective function against reactive oxygen species is defined. Emphasis is also given to the role of androgens in controlling the secretory activity of boar seminal vesicles. *Reproductive Biology 2002 2 (3): 243-266*

Key words: boar, semen, seminal plasma, seminal vesicles, plasma membrane, proteins

INTRODUCTION

Boar seminal vesicles are paired and large compared with the other accessory sex glands. A detailed description of morphology of the seminal vesicles at the light and electron microscopic levels has been presented

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Aumüller and Seitz [1]. The epithelium of these glands is typical of a secretory organ which consists of basal, endocrine cells with specialized surface [10]. It is generally accepted that the expulsion of seminal vesicles' contents during ejaculation is largely under adrenergic control, and norepinephrine is the major excitatory neurotransmitter [16].

Seminal vesicle proteins: physiological functions

The composition of seminal vesicles has been investigated more thoroughly in the boar than in other species. A study of seminal vesicle proteins has attracted an increased interest and a number of new results have been published, most notably by Boursnell and co-workers of Cambridge University (tab. 1). These authors have presented some of the

Tab. 1. Brief history of the early research on boar seminal vesicle proteins

Finding	References
• chromatographical isolation (Sephadex G-200) of two major protein fractions (fraction A, pI 8.2, 80-90% of the total proteins);	Boursnell et al. [3, 6] Boursnell & Nelson [4]
• presence of a powerful haemagglutinin identified as a mixture of proteins (protein H) with a high isoelectric point (pH 9.5);	Boursnell &Combs [5] Boursnell & Briggs [7] Lavon et al. [36] Nelson & Boursnell [40]
• a high level of Zn^{2+} concentration (137 mg/ml) in seminal vesicle secretion; • finding correlations between Zn^{2+} and citrate and total nitrogen of the seminal vesicle secretion;	Boursnell et al. [8]
• discovery of the phenomena regarding Zn^{2+}, temperature and pH dependent precipitation of boar seminal plasma; • proteins precipitated by Zn^{2+} (ZPP) and protein H are similar seminal vesicle proteins	Boursnell & Roberts [9] Nelson and Boursnell [40] Roberts et al. [44-45]
• gel disc electrophoresis and isoelectric focusing on polyacrylamide gel of many discrete protein components of seminal vesicle secretion; • significant differences between proteins secreted by the seminal vesicles and epididymides	Lavon & Boursnell [34] Lavon et al. [35] Lavon et al. [36]

cognitive and practical aspects of the functions of boar seminal vesicle proteins. Remarkably, the occurrence of a high concentration of basic proteins of seminal vesicle origin (proteins precipitated by zinc and haemaglutinin) in boar seminal plasma has been a subject of great interest. The absorption of the discussed protein on boar sperm membrane, particularly intensive below 10°C, was accompanied by an irreversible loss of motility and increased susceptibility of the plasma membrane to cold shock [44, 45]. A number of studies have been conducted in order to solve this problem. It was shown that the surgical removal of the seminal vesicles or binding of the basic protein by Zn^{2+} ions by preincubation of seminal plasma with egg yolk or phospholipid fraction did not give conceivable results regarding the fertilizing ability of stored liquid boar semen at different temperatures. Even though liquid storage (16°C) or deep freezing of spermatozoa from boars without seminal plasma give slightly higher conception rates than those from intact animals, the differences were not statistically significant [40]. Boursnell and co-authors reported for the first time, that boar seminal vesicles are involved in fertility but cannot be considered to be totally responsible for it [8, 9]. Secretions of these glands appear to protect the spermatozoa and enhance their lifespan and fertilizing capacity. Moreover, in recent years much knowledge has been accumulated on the nature and biochemical properties of seminal vesicle proteins. In the boar, the major proteins that have been purified originate from the sperm plasma membrane and seminal vesicle fluid.

The multifunctional properties of boar seminal vesicle proteins seem to be facilitated by their structure and molecular mechanisms enabling their interactions with various substances. Important roles of these proteins in the reproductive processes are as follows:

- physiological modulations of sperm function during transport in the female reproductive tract and egg fertilization;
- inhibitory and stabilizing activity against enzyme systems and sperm chromatin;
- immunosuppressive and immunomodulation properties;
- participation in the defense mechanism of spermatozoa against reactive oxygen species;
- enzyme activity assists in sperm metabolism.

Many cell surface modifications occur at ejaculation and during the transport of spermatozoa within the female reproductive tract. Sperm coating

and uncoating by proteins are claimed to be mainly associated with the processes of decapacitation and capacitation, respectively. It is generally accepted that the binding of factors from seminal plasma to spermatozoa stabilizes the components of the plasma membrane, masks antigens exposed to cell surface and prevents a premature acrosome reaction.

Literature on sperm-coating proteins in boar semen seems to be exceptionally abundant. Russell et al. [48] used the two-dimensional gel electrophoresis to analyze polypeptide profiles of detergent-solubilized sperm plasma membrane in epididymal fluid, accessory gland secretions, and seminal plasma of the boar. When SDS-PAGE patterns of plasma membranes from cauda epididymidal sperm were compared with those of plasma membrane from the same cells incubated in seminal vesicle fluid, large amounts of low-molecular weight proteins (approximately 20 kDa) were added by seminal vesicle secretion. It is interesting that the fluid of the boar seminal vesicle shows about 150 acidic and neutral-range polypeptides spread throughout a spectrum of molecular weights. Most major polypeptides migrate towards neutrality or in the highly basic region during two-demensional electrophoresis.

Boar seminal plasma is a highly diluted form of seminal vesicle secretion; diluted to the extent that most polypeptides from other glands and the epididymis are not in sufficient concentrations to be noted even with the highly sensitive silver-staining technique [48]. Lavon and Boursnell [3, 4], utilizing isoelectric focusing on polyacrylamide gel, estimated that 80-90% of the protein in seminal plasma was derived from seminal vesicles with the remaining detectable protein from the epididymal fluid and prostate. Recent evidence has shown that some seminal vesicle proteins (especially low-molecular glycoprotein) have a role in stabilizing the plasma membrane and acrosome to promote survival in the female reproductive tract so that spermatozoa can arrive at the site of fertilization in a fully competent state [25].

The initial interaction between the gametes is a molecular receptor--mediated process which takes place at the surface of the egg coat, especially on the zona pellucida. The following structures are involved in binding of the capacitated spermatozoa to the zona pellucida of a homologous oocyte:

- numerous chains of O-linked oligosaccharides (≈3.9kDa) of glycoprotein ZP-3 present in zona pellucida which act as primary ligands locating specific spermatozoon receptors and binding with them;

- the so-called zona pellucida-binding proteins located on the plasma membrane of spermatozoa act as receptors.

In general, the zona pellucida-binding proteins of spermatozoa can be divided into two broad categories, those which have enzymatic activity, and those which do not have enzymatic activity but do have lectin properties. Töpfer-Petersen and colleagues [61-63] have reported a variety of putative zona pellucida-binding proteins on spermatozoa in a number of mammalian species. These studies should be considered ground breaking as regards the pivotal roles of the zona pellucida-binding proteins in boars. The authors have detected a family of sperm-associated low-molecular weight lectins in boar, known as spermadhesins. This discovery has been of great interest in molecular biology of fertility process in mammals.

There is abundant literature regarding the structure, biochemical properties, and physiological functions of spermadhesins. Some of the results of previous studies are presented in table 2. These studies emphasize the importance of seminal vesicle proteins in the molecular mechanisms of the sperm plasma membrane and the gradual exposure of protein receptor systems in the sperm plasma membrane, particularly during capacitation. Some of these proteins are structural and sperm-modulating proteins or transport- and immunomodulating proteins. Our team has undertaken complex studies on the system of boar seminal vesicle proteins. Some of the peptide and protein substances isolated in the course of our studies as well as descriptions of their biochemical and biological properties are shown in table 3. The discussed substances seem to be multifunctional with respect to their biological role.

We have been able to purify a unique protein from boar seminal vesicle fluid or seminal plasma defined as glycoprotein complex 54 kDa (Gp54), using modern chromatographic techniques, mainly Chelating Sepharose Fast Flow columns bound with zinc ions. The Gp54, a heterogeneous plasmatic protein, secreted by the seminal vesicle glands consists of three proteins (15 kDa; 16 kDa; 18 kDa) that belong to the family of spermadhesins [23, 54]. The 54 kDa glycoprotein is characterized by lipoprotein adhesion properties and antitrypsin activity, and may act as a decapacitation factor that must be removed, modified or masked before spermatozoa can undergo the acrosome reaction. Our study has also demonstrated that this glycoprotein suppresses some immunologic functions *in vitro*. The 54 kDa glycoprotein suppresses spontaneous and pokeweed mitogen (PWM) stimulation of

lymphocyte blastogenesis as well as binding of porcine IgG. We have also detected that the 54 kDa glycoprotein may be one of the immunosuppressive factors of boar seminal plasma [54].

An immunoglobulin-binding protein was reported in human and boar seminal plasma [26, 32, 38]. It is important to note that the 16 kDa protein, a component of human seminal plasma which binds specific subclasses of immunoglobulins, was found to be identical with the protein factor that specifically suppressed PWM-induced lymphocyte blastogenesis [37].

*Tab. 2***.** Spermadhesins of boar seminal vesicles

Spermadhesins	**References**
• major secretory products of the seminal vesicles in boars (0.6 to 7.0 mg/ml of seminal vesicle fluid); • 111-113 aminoacids, two conserved disulphide bonds between the nearest cysteine residues, 40-60% aminoacids sequence identity;	Calvete et al. [13-14] Dostalova et al. [17] Töpfer-Petersen et al. [62]
• spermadhesin AWN is synthesized by the rete testis, tubuli recti, and seminal vesicles; • spermadhesin AWN has been detected on spermatozoa in the sow genital tract and on plasmalemma remnants of spermatozoa bound to the zona pellucida in vivo; • glycoform AWN-1 may be responsible for the ability of epididymal spermatozoa to fertilize mature oocytes;	Calvete et al. [11-12] Dostalova et al. [18] Rodriguez-Martinez [46] Töpfer-Petersen et al. [62]
• spermadhesins, zona pellucida glycoproteins, serine protein inhibitors and heparin are implicated in similar molecular binding activity;	Sanz et al. [49-50]
• spermadhesins may perform biological functions in gamete interactions, (90% of the spermadhesins released from ejaculated spermatozoa during in vitro capacitation may act as decapacitation factors)	Dostalova et al. [17] Töpfer-Petersen & Calvete [61]
• glycosylation may be a mechanism for modulating the ligand-binding properties of spermadhesins	Sanz et al. [49] Calvete et al. [11-13] Töpfer-Petersen [63]

We have earlier reported that the 54 kDa glycoprotein has multiple functions [22]. For example, one of the biological properties of this glycoprotein--complex is that it reduces motility of spermatozoa of individual boars in a dose-dependent manner. It is interesting to note that immunoblotting assay with specific antibodies confirmed that an anionic peptide (sperm motility inhibiting peptide-SMIF) is a component of the 15 kDa protein [27]. This suggests that 54 kDa glycoprotein is the precursor of SMIF. The biological functions of SMIF are presented in table 4.

It is necessary to underline that SMIF causes immediate inhibition of sperm motility without affecting the integrity and permeability of the plasma membrane, as assessed by *in vitro* fluorescent probes [27]. This peptide induces dramatic changes in the molecular organization of the plasma membrane, thus affecting the energy charge and biochemical functions of the sperm structure. When taken into consideration some of the properties of SMIF, such as its protective action against lipid peroxidation, antitrypsin activity, as well as the suppression of spontaneous decondensation of sperm chromatin, this peptide seems to play an important role in the regulation

Tab. 3. Protein and peptide substances isolated from boar vesicular fluid

Substances	**Secretion**	**References**
Zn^{2+} - ion dependent protein	seminal vesicle	Strzeżek & Hopfer [51] Strzeżek et al. [52]
Sperm motility inhibiting factor (SMIF)	seminal vesicle	Kordan et al. [27] Strzeżek et al. [53] Velev et al. [65]
High-molecular proteinase inhibitor	seminal vesicle, epididymis	Strzeżek & Torska [59] Torska & Strzeżek [60]
54k Da glycoprotein	seminal vesicle	Hołody et al. [22] Hołody & Strzeżek [23] Kordan et al. [28] Plucienniczak et al. [42] Strzeżek & Holody [54]
Phosphotyrosine acid phosphatase	seminal vesicle	Wysocki & Strzeżek [66, 68]
Platelet-activating factor acetylhydrolase (PAF-AH)	seminal vesicle prostate	Kordan et al. [29] Kordan & Strzeżek [30]

of reproductive processes in the boar. As a component of the adhesive proteins of boar seminal plasma, it may act as a decapacitation factor which stabilizes receptors necessary for sperm-oocyte interaction. Our immunologic studies have shown that both Gp54 and SMIF bind selectively to the receptors of the plasma membrane overlying the acrosome and mid-piece regions of spermatozoa [28]. It has been confirmed that SMIF inhibits the acrosome reaction induced by calcium ionophore, A 23187. In order to investigate the biological roles of Gp54 and SMIF, an *in vitro* test was conducted to evaluate the ability of these antigens and their antibodies to modulate boar spermatozoa binding to the homologous zona pellucida.

The effect of Gp54 on the percentage of spermatozoa bound to the zona pellucida is presented in table. 5. As regards the pre-treatment of oocytes with glycoprotein Gp54 (containing spermadhesin), the mean number of bound spermatozoa per oocyte was markedly reduced when compared to

Tab. 4. Characteristics of sperm motility inhibiting factor (SMIF); Kordan et al. [28], Strzeżek et al. [43]

Biochemical characteristics	**Biological activity**
• molecular weight 5.7 kDa • thermal stability (10 min at 100ºC) • isoelectric point (pI) 5.3 • amino acid composition: glutamic acid (predominant), glycine, serine, aspartic acid, alanine, threonine • inhibitory activity at pH 6.5-8.0 • proteolytic enzyme (trypsin, chymotrypsin) resistant	• antigen of boar seminal plasma (component of 54 kDa gycoprotein complex, especially spermadhesin DQH) • species specific antigen determinant • immunomodulation factor • inhibition of the acrosome reaction • reduction of number of spermatozoa bound to the plasmalemma • species unspecific inhibitory influence on sperm motility • reduction of sperm energy charge • increase in AspAT leakage • suppression of lipid peroxidation of sperm plasmalemma • stabilization of sperm chromatin • antibacterial properties towards Gram-positive strains • lysozyme – like activity

the control. These data indicate that Gp54 competes with spermatozoa for binding sites on the zona pellucida. When the capacitated spermatozoa were pre-treated with Gp54 before co-incubation with oocyte, the number of spermatozoa tightly bound to zona pellucida was also significantly reduced. This may be related to the decapacitating effect of Gp54, which causes a reduction in the number of spermatozoa tightly bound to the zona pellucida. Furthermore, pre-treatment of capacitated spermatozoa with polyclonal antibodies against Gp54 reduced the number of bound spermatozoa, which was more marked when the IgG serum fraction was used. These results clearly show that the antibodies against this fraction can disturb the process of egg fertilization.

The presented biological function of Gp54 resulted from its hetero-geneous composition of glycosidic form of spermadhesins. Our studies were directed mainly towards the 15 kDa component because of its immuno-suppressive and adhesion properties. We have isolated complementary DNA (cDNA), using modern techniques of molecular biology, to characterize the

Tab. 5. The effect of Gp-54 on the percentage of spermatozoa bound to zona pellucida; Kordan et al. [28]

Incubation variant	No. of oocytes examined	No. of spermatozoa bound to oocyte	*ZPBI
Preincubation of oocytes with Gp-54	17	3.76 ± 0.82c	7.32
Preincubation of spermatozoa with Gp-54	32	5.28 ± 0.73c	16.27
Preincubation of capacitated spermatozoa with anti-Gp-54 serum	22	13.18 ± 1.63b	25.63
Preincubation of capacitated spermatozoa with anti-Gp-54 IgE	17	9.47 ± 0.98b	18.42
Incubation of gametes in TBM (control)	26	51.42 ± 6.05a	

[a,b,c] Means within the column with the same superscripts are not different significantly at p<0.005; *ZPBI - zona pellucida binding index.

primary nucleotide sequences and structure of the 15 kDa protein, and to analyze the amino acid sequence of this protein. These findings demonstrate that, besides AQN-3 and AWN spermadhesins reported by the Töpfer-Petersen group, DQH - protein showing homology with a leukocyte adhesion--inducing protein (pAIF-1) described by Hadjisavas et al. [21] - and pB1 protein [15] are part of the 54 kDa glycoprotein-complex.

The cDNA sequence encodes a 130-amino acid-long polypeptide which contains a 25 - amino-acid long signal peptide (fig. 1). It is interesting that the protein contains two fibronectin type II domains. It should be noted that fibronectin with dimeric structure is an adhesion glycoprotein, which has a

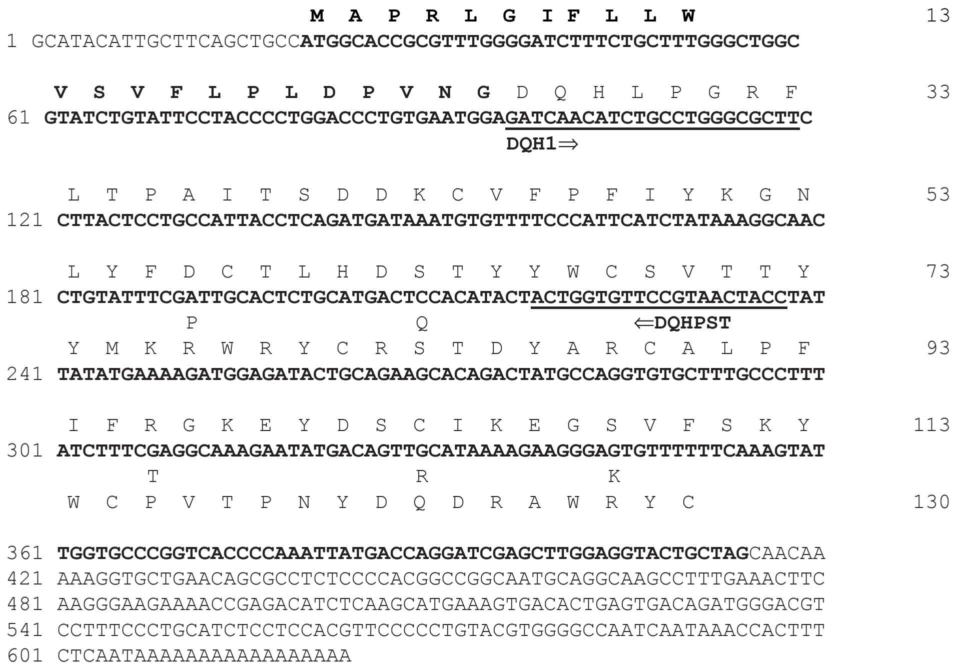

Fig.1. Complementary DNA of boar seminal plasma protein DQH (pB1) and its amino acid sequence derived from cDNA translation. The DNA coding sequence is shown in bold. The putative leader peptide is in italic bold. The differences in amino acid sequence are doubly underlined. The variants established by Calvete et al. [15] are placed above the sequence determined by Płucienniczak et al. [12]. The putative polyadenylation site is shadowed. Numbering: left and right margins, nucleotides and amino acids, respectively.

strong affinity for glycosaminoglycans and gangliosides, and binds receptors of different cells. We have determined the sequence for DQH protein (different from that presented by Calvete and co-workers for pB1 protein in 5 positions), which occurs in boar seminal vesicles, and consists of a large family of cell and matrix adhesion proteins, including boar seminal plasma proteins. Bezouška et al. [2] reported that these differences might reflect the existence of two different, albeit closely related proteins (pB1 and DQH) resulting probably from allelic polymorphism in the gene encoding the porcine pB1 sperm surface protein.

Phosphotyrosine protein acid phosphatase - its significant role

Recently, we have detected a high activity of phosphotyrosine acid phosphatase in the fluid and tissue of boar seminal vesicle glands. In boar seminal plasma, this enzyme has four molecular forms with different electrophoretic profiles, which originate from the epididymal, prostatic and seminal vesicle fluids [66]. However, in the seminal vesicle fluid only one molecular form of acid phosphatase (approximately 6% of the total acid phosphatase activities) occurs. This enzyme is termed phosphotyrosine protein acid phosphatase because of its high affinity for phosphorylated substrates (phosphotyrosine). Some of the biochemical properties of this enzyme, which was purified about 500 times from the seminal vesicle fluid, are shown in table 6.

Phosphotyrosine protein acid phosphatase catalyses the hydrolysis of phosphotyrosine residues in protein, and is involved in the control of cell proliferation and differentiation. Nguyen et al. [41] reported that the activity of prostatic acid phosphatase in the serum of a patient with prostatic cancer is a specific phosphotyrosine acid phosphatase. Hence, assay of prostatic acid phosphatase, which is antigenically distinct from other isoenzymes of acid phosphatase in serum, has been used in the diagnostic and monitoring of prostatic cancer in people. It has been reported that phosphotyrosine phosphatase seems to be an important anticarcinogenic factor. In normal cells, phosphotyrosine level is 0.003% of that of phosphoamino acid in proteins, however, its level rises about 5-10 times in carcinogenic cells. This suggests that the reduction in phosphotyrosine phosphatase activity in the tissue may be related with carcinogenic changes. Phosphotyrosine acid phosphatase is glycoprotein and is resistant to desialylation. Moreover,

Zn^{2+} ions, components of some seminal vesicle proteins, do not have any stimulatory effect on the enzyme activity. No changes in the enzyme activity were observed in the presence of EDTA (ion chelator), thus indicating that phosphotyrosine phosphatase is not a metalloprotein. It seems highly probable that boar seminal vesicles, with regard to their physiological role, have an autonomic enzyme system in the form of phosphotyrosine protein acid phosphatase, which protects these glands against carcinogenic development.

The regulation of protein tyrosine phosphorylation, which is generally modulated by tyrosine kinases and phosphatases, is crucial to sperm functioning since protein tyrosine phosphorylation is involved in epididymal maturation, capacitation, acrosome reaction and fertilization [43, 64, 67]. Recently, Flesh et al. [19] have confirmed that in the case of boar sperm, capacitation is induced by tyrosine phosphorylation of three proteins, which coincides with an increase in plasma membrane fluidity. It can be suggested that, besides the plasma membrane binding of phosphotyrosine phosphatase, the seminal vesicle enzyme, which belongs to the group of coating protein

Tab. 6. Biochemical properties of phosphotyrosine protein acid phosphatase isolated from fluid of boar seminal vesicles; Wysocki and Strzeżek [68]

Biochemical properties of phosphotyrosine protein acid phosphatase	
molecular weight	approx. 41kDa
isoelectric point (pI)	7.1
optimum pH for hydrolysis of p-nitrophenyl phosphate	5.5
K_m values for substrates: phosphotyrosine tyrosine-phosphorylated oligopeptides of gastrin C-terminal fragment of hirudin p-nitrophenyl phosphate phenyl phosphate	0.37 x 10-3M 0.00032 x 10-3M 0.0075 x 10-3M 0.87 x 10-3M 1.62 x 10-3M
inhibitors	vanadate and molybdate ions, dephostatin
high thermal stability	70°C
resistant to desialylation	
non-metalloprotein, non-stimulatory effect of EDTA and Zn2+ions on its activity	

of spermatozoa, takes part in the discussed process and may act as a decapacitation factor. The release of coating proteins (including spermadhesins and acid phosphotyrosine phosphatase) accompanies capacitation and may induce the acrosome reaction, but, this hypothesis needs further investigation.

Platelet activating factor acetylhydrolase (PAF-AH) - properties and functions

Platelet activating factor, PAF, (1-O-alkyl-2-acetyl-*sn*-glycero3-phosphorylcholine) has been detected in mammalian sperm of only several animal species [31]. Considerable evidence has been accumulated suggesting that PAF may play a key regulatory role in sperm biological functions, such as sperm motility, capacitation and acrosome reaction [31]. Moreover, PAF-induced activation of tyrosine kinase, which results in tyrosine phosphorylation of specific plasma membrane proteins, is one of the mechanisms regulating the sperm biological functions [33]. Roudebush and Diehl [47] demonstrated differences in the mean levels of PAF in ejaculates of boars that differed in fertility (farrowing rate data).

It has been reported that PAF enhanced sperm motility [30]. These results demonstrated that the addition of exogenous PAF to stored liquid boar semen at 5° and 16°C enhanced sperm motility and viability. These effects were dose-dependent, as shown by the addition of higher PAF concentrations ($>1x10^{-6}M$) to stored liquid boar semen. Higher PAF concentrations resulted in an increased leakage of cytoplasmic aspartate aminotransferase indicating changes in the sperm plasma membrane integrity at the mid-piece region. Fluorescent microscopy also showed that PAF affected the plasma membrane overlying the acrosome region, as indicated in an increased number of membrane-damaged spermatozoa assessed by fluorochrome Hoechst 33258 (H33258). It is interesting to note that the deacylated form of PAF (lyso-PAF) appeared to have a detrimental effect on motility of boar sperm stored at 5°C and 16°C.

The concentration of sperm PAF is governed by its rate of synthesis and degradation. Degradation of PAF is generally limited by the activity of PAF-acetylhydrolase (EC 3.1.1.47), which is the major metabolic enzyme for PAF. The activity of PAF-acetylhydrolase has been detected in human seminal plasma as well as in the seminal plasma of several domestic animal species [24]. In contrast to stallion seminal plasma, PAF-AH activity in

seminal plasma of bull, rabbit and rooster was calcium-independent. The PAF-AH activity in stallion seminal plasma was partially inhibited, whereas in rooster seminal plasma it was stimulated by EDTA [24]. Such differences in PAF-AH activity in seminal plasma might be related to species differences in PAF production and regulation by sperm or by accessory sex gland secretions [39].

More recently, our laboratory has purified (44-fold) and characterized PAF-AH from boar seminal plasma [29]. Some of the biochemical and biological properties of the purified PAF-AH are presented in table 7. The large molecular weight of the purified protein (310 kDa) may be related to its high degree of glycosylation and susceptibility of seminal plasma proteins for aggregation, which can affect the efficiency of the enzyme purification. Calcium ion dependence of PAF-AH activity in boar seminal plasma was similar to that observed for the enzyme activity in stallion seminal plasma [24].

Platelet-activating factor acetylhydrolase in boar seminal plasma is believed to originate from the seminal vesicles and prostate. In addition, biochemical methods and indirect immunofluorescence technique have

Tab. 7. Biochemical properties of PAF-AH isolated from boar seminal plasma; Kordan [29]

Biochemical properties of PAF-AH	
molecular weight	approx. 310kDa
isoelectric point (pI)	4.58
optimum pH for hydrolysis of PAF	7.3
Km values for PAF	4.14 x 10^{-6}M
inhibitors: strong weak	p-bromophenacylbromide (p-BPB) EGTA, Zn^{2+}ion
activator	Ca^{2+}
high thermal stability	90°C
secretory source	seminal vesicles, prostate
strong affinity for sperm plasma membrane	

shown that PAF-AH binds to the plasma membrane of boar spermatozoa. It has been suggested that the negative correlation found between PAF-AH activity and sperm concentration might indicate that a large quantity of the enzyme is bound to the sperm plasma membrane [29].

Boar spermatozoa are characterized by a high proportion of unique phospholipids which are rich in polyunsaturated fatty acids. The double bonds are the primary site of free radical attack during the initiation phase of lipid peroxidation. Moreover, one of the consequences of membrane lipid peroxidation is phospholipid fragmentation that enhances substrate availability for PAF-AH activity. This may attribute to the relatively high level of PAF-AH activity in boar seminal plasma, which can help to protect boar sperm from peroxidative damage as well as regulating PAF content and activity.

Antioxidant properties of seminal vesicle fluid

Protection against reactive oxygen species is provided by enzyme degradation, scavenging by antioxidants and molecular repair. The sperm lack the biosynthetic machinery required to repair peroxidative damage once it has occurred [57]. It is interesting to note that boar spermatozoa, in comparison with other animal species, have insufficient protective enzymes (tab. 8). The enzymes of the glutathione cycle are remnant and catalase does not occur in boar spermatozoa. Furthermore, the activity of superoxide

Tab. 8. Enzyme activity against reactive oxygen species of boar spermatozoa (mean ± SD); Strzeżek et al.[1] [57]

Enzyme	Activity	
	U/mg protein	U/10^9 spermatozoa
Superoxide dismutase	22.94 ± 14.63	21.46± 9.56
Glutathione peroxidase	0.21 ± 0.01	0.28 ± 0.09
Glutathione reductase	0.05 ± 0.02	0.07 ± 0.01
Catalase	non-detectable	non-detectable

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

dismutase (SOD) is represented by one molecular form in spermatozoa and two molecular forms in seminal plasma, probably originated from the seminal vesicle secretions[1]. The activity of SOD is limited only to the dismutation of superoxide anion, which accompanies the formation of hydrogen peroxide. This probably indicates that the protective function against reactive oxygen species in boar semen may be provided by the low and high-molecular weight antioxidants.

The seminal vesicle glands are the major source of low molecular weight antioxidants in the boar. Ergothioneine content in vesicular fluid is 25.1 ± 6.8 mg/100ml, whereas that of L-glutathione and L-ascorbic acid is 75.7 ± 14.4 and 9.8 ± 1.3 mg/100ml, respectively. These antioxidants are classified as preventive or chain breaking, hydrophilic antioxidants of the seminal plasma. Ergothioneine (ERT) and glutathione (GSH) may exert a specific physiological role in boar semen (correlation coefficient between these two components was $r = 0.34$; $p<0.001$). This role has been mediated by reducing the sulphydryl group which consequently has a protective influence on the spermatozoa and plasmatic enzymes[2].

Table 9 presents the content of total protein, ergothioneine and glutathione of boar seminal plasma. Sulphur-containing antioxidants, ERT and GSH, occur, as usual, in similar concentrations. It is interesting that a highly statistically significant correlation coefficient was observed between ERT and thiol content in seminal plasma ($r = 0.40$; $p<0.001$). It is possible that ERT oxidized in the process of reducing some oxidants can be reduced back to its thiol/thione form by the reaction with reduced glutathione. The correlation coefficients of the total protein content or antiperoxidant activity (measured as percentage inhibition of malondialdehyde production) and some antioxidants parameters of seminal plasma are shown in table 10. It is interesting to note that the majority of these coefficients were positive and highly significant. The dominant role of the seminal vesicles in influencing the antiperoxidant capacity of boar seminal plasma is

[1]Strzeżek J Juklińska M Szreder T 2002 Superoxide dismutase (SOD) in boar semen (In Polish). *Proceedings of Third Conference on the Society of Biology of Reproduction* 4-7 September 2002, Międzyzdroje, p. 88.

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

evident. A relationship exists between the various protein ranges and total protein content, as well as with low-molecular antioxidants and seminal plasma inhibiting properties of lipid peroxidation induced by ascorbate-Fe^{2+} ions [58, 59].

Our earlier work based on the administration of atropine, a parasympathetic--blocking agent, showed that the antiperoxidant properties of seminal vesicle fluid probably function together with components of the epididymal fluid [55]. It should be underlined that atropine severely inhibits the secretory

Tab. 9. Total protein content, antioxidant concentrations and antiperoxidant activity of boar seminal plasma; Strzeżek et al. [57]

Parameters (n=132)	**mean ± SD**
Total protein content (mg/ml)	53.8 ± 23.9
L-ascorbic acid (mg/100ml)	2.9 ± 1.6
L-ergothioneine (mg/100ml) (µg/10^9 spermatozoa)	6.7 ± 4.7 189.6 ± 121.5
Glutathione (after protein precipitation) (mg/100ml) (µmoles)	 5.7 ± 1.4 185.8 ± 46.7
Thiol content (µmoles) (µM/100mg protein)	247.7 ± 76.6 1.1 ± 3.7
Antiperoxidant activity (%)	33.3 ± 18.0

Tab. 10. Correlation coefficients of total protein content, antiperoxidant activity and antioxidant parameters of boar seminal plasma; Strzeżek et al.[1] [57]

Parameters	**Total protein content**	**Antiperoxidant activity (inhibitory effect on lipid peroxidation)**
L-ascorbic acid	0.50***	0.28**
L-ergothioneine	0.34**	0.41***
Thiol content	0.68***	0.61***
Total protein content	-	0.81**

**$p \le 0.01$; ***$p < 0.01$

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

activity of the bulbo-urethral glands when compared with that of the seminal vesicles and epididymides. However, the mechanism by which atropine affects the secretory activity of the accessory sex glands has not been elucidated. Moreover, the administration of different doses of atropine caused a marked increase in antioxidant level of boar seminal plasma. This phenomenon may be of particular importance since it shows that the antioxidant capacity is derived from a number of components such as enzymes, proteins and metabolites secreted by the seminal vesicle glands and probably by the epididymis. These components have different contributions to the several functions of the antioxidant system of boar seminal plasma. Nevertheless, the high correlation between the antioxidant and total protein content in the seminal plasma underline an important role of the seminal vesicle secretions.

Hormonal dependence of boar seminal vesicle glands

Androgens are essential hormones for the function of boar seminal vesicles. Our study based on the daily administration of a low dosage of synthetic antiandrogen, cyproterone acetate (CPA), showed that this treatment resulted in a significant reduction in luteinizing hormone, testosterone, oestrone sulphate and oestrone concentration in blood plasma [20, 56, 58]. It was found that CPA caused a gradual reduction in libido and potency, and was responsible for permanent disturbances in the parameters of semen quality and quantity [58]. However, no inhibitory action on spermatogenic activity was observed. Prolonged CPA treatment induced a severe impairment in the content of selected non-protein components of the seminal plasma, particularly biochemical markers of the seminal vesicle glands such as fructose, citric acid, and Zn^{2+} ions (tab. 11). Some of the biochemical properties of the seminal plasma proteins were disturbed during antiandrogenesis [56].

In addition to changes in the activity and electrophoretic profiles of the molecular forms of acid and alkaline phosphatase, disturbances in the chromatographic profile of plasmatic protein were demonstrated. The autolytic process or synthesis of new polypeptides observed after a longer treatment with CPA was manifested in the appearance of two to four new polypeptides with high molecular weight and two polypeptides with a medium and low molecular weight. Furthermore, immunoelectrophoretic

Tab. 11. Effect of cyproterone acetate (CPA) on quantity and quality of boar semen (means±SD); Fraser et al. [20], Strzeżek et al. [56, 58]

Parameters	Pre-CPA treatment (control) (n=40)	CPA treatment (60d) (n=45)
Semen quantity and quality		
• total volume (ml)	236.4 ± 53.2	119.3 ± 55.8**
• gel free volume (ml)	170.1 ± 33.3	85.2 ± 39.9*
• sperm concentration	444.2 ± 176.6	800.0 ± 476.5**
• no. of sperm per ejaculate (x 10^9)	73.9 ± 30.4	58.4 ± 23.1*
• motility	75.7 ± 1.9	71.0 ± 6.8**
• total abnormal sperm	22.6 ± 9.2	68.6 ± 30.2***
proximal droplets	0	34.2 ± 21.9***
distal droplets	7.2 ± 7.6	11.7 ± 15.3**
• agglutinated spermatozoa (%)	6 - 10	30 - 40
Biochemical parameters		
• pH	7.9 ± 0.2	8.0 ± 0.2**
• total protein (mg/ml)	26.5 ± 10.9	38.4 ± 19.4**
• fructose (mg%)	12.7 ± 6.0	8.2 ± 6.8*
• citric acid (mg%)	171.4 ± 87.0	119.4 ± 89.9*
• zinc (mg%)	2.8 ± 1.4	2.1 ± 1.8*

significant from controls at *p≤0.05; **p≤0.01; ***p≤0.001

studies showed that the antigenic structure of boar seminal plasma was affected [55].

It should be noted that the inhibition of androgen synthesis in the testis disturbs the distribution of particular secretions of epididymis and seminal vesicle glands and changes their secretion effectiveness. Consequently, there were disturbances in the physiological functions of the protein spermatozoa systems as well as the stabilization mechanism of sperm chromatin, which corresponds with changes in Zn^{2+} ions-protein secretion of boar seminal vesicle glands.

CONCLUSIONS

The seminal vesicles are involved in the control of fertility. It is very difficult to study the specific functions of seminal vesicles. The active factors in boar seminal plasma most frequently are a result of the interactions taking place among different products of accessory sex glands (prostate, epididymis, seminal

vesicles). In the boar, seminal plasma secretions of the seminal vesicle glands are dominant. Changes in these secretions may cause disturbances in the concentration of active factors produced from precursors present in vesicular fluid. As a consequence, the biological functions of sperm may be disturbed since the interaction of seminal plasma proteins with receptors on the plasma membrane of spermatozoa determine its fertilizing ability. The properties of the seminal plasma depend on the equilibration of the secretions of different accessory sex glands of the boar reproductive tract. Isolation and detailed examination of components of the seminal vesicle fluid, particularly protein substances, deserves more scientific attention.

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