Application of biochemical markers for identification of biological properties of animal semen

Leyland Fraser², Paweł Wysoki, Andrzej Ciereszko³, Grażyna Plucienniczak⁴, Mariola Kotlowska³, Władysław Kordan², Mariola Wojtczak³, Grzegorz Dietrich³, Jerzy Strzeżek¹,²
²Department of Animal Biochemistry and Biotechnology, University of Warmia and Mazury in Olsztyn; ³Institute of Animal Reproduction and Food Research, Semen Biology Group, Polish Academy of Sciences, Olsztyn; ⁴Institute of Biotechnology and Antibiotics, Warsaw, Poland

SUMMARY

The use of biochemical markers for identification of biological properties of semen will help to develop new criteria that are accurate and objective in predicting and improving male fertility. Understanding and controlling the mechanisms involved in fertility is a key challenge, which is of fundamental importance in successful animal reproductive performance. Moreover, unraveling the unique molecular mechanism associated with sperm function might have considerable diagnostic value in the evaluation of male infertility. This review offered insights into some recent achievements and provided perspectives for possible applications of the biochemical markers of semen. Reproductive Biology 2006 6 Suppl. 1:5–20.

Key words: spermatozoa, cryopreservation, DNA fragmentation, comet assay, acid phosphatase, PAF-AH

¹Corresponding author: Department of Animal Biochemistry and Biotechnology, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-718 Olsztyn, Poland; e-mail: kbz@uwm.edu.pl

Copyright © 2006 by the Society for Biology of Reproduction
INTRODUCTION

Over the last decades, rapid advances in reproductive molecular biology have resulted in the development of numerous techniques to assess semen quality. An understanding of the molecular mechanisms underlying male infertility is being vigorously pursued in order to improve assisted reproduction techniques (ARTs). However, the use of broadly applicable markers to identify the biological properties of semen may provide specific information about the sperm functional disturbances and may aid in the diagnosis of male infertility.

One of the research areas that has been studied intensely during the past decade as a cause of male infertility is the integrity of DNA in the nucleus of mature ejaculated spermatozoa. Fertilization with DNA-damaged spermatozoa may lead to paternal transmission of defective genetic material with adverse consequences for embryonic development [1]. The DNA-damaged spermatozoa can form pronuclei following fertilization and allow for normal embryo development and this has led investigators to recommend the assessment of sperm DNA damage as a part of the assisted reproductive programme.

There is increasing evidence for the multifunctional nature of proteins and it is possible that enzymes may have different functions in the male reproductive tract. Recent research has been shown that acid phosphatase (AcP) and platelet activating factor, PAF, (1-O-alkyl-2-acetyl-sn-glycero3-phosphorylcholine) of boar seminal plasma can modulate sperm physiology [19, 28]. Acid phosphatase originating in the epididymis is implicated in the processes of phosphorylation-dephosphorylation of proteins, which accompany post-testicular sperm maturation. Moreover, the synthesis and degradation of PAF, a naturally occurring membrane phospholipid compound, are controlled by the activity of membrane-bound cytosolic and extracellular enzymes, particularly platelet-activating factor acetylhydrolase, PAF-AH (EC 3.1.1.47; [2, 17]). The results presented in this review gave an overview on the application of biochemical markers for identification of biological properties of semen. These markers include analysis of sperm DNA integrity, using measurements of the
comet assay, epididymal acid phosphatase and platelet-activating factor acetylhydrolase.

EVALUATION OF SPERM DNA INTEGRITY FOLLOWING SEMEN PRESERVATION AND INDUCED ENVIRONMENTAL STRESS

Use of the modified neutral comet assay to assess DNA integrity of boar spermatozoa

Mammalian protamines are very rich in arginine and possess cysteine residues that form disulfide cross-links, conferring an extremely high degree of compactness to mature sperm chromatin, which is important to protect the sperm genome from external stresses such as oxidation or temperature elevation in the female reproductive tract. While in most mammals examined so far only one protamine variant is present in spermatozoa, two protamines (P1 and P2) were found in mouse and human [4]. Evidence has been shown that P1 in the boar is superseded by P2 and is functionally deficient due to significant changes within its primary structure [21]. However, the protamines and associated sulfhydryl groups may have an important role in the process of decondensation during fertilization in the pig.

The comet assay or the single cell gel electrophoresis (SCGE) is a visual technique used to assess DNA integrity in individual cells by measuring the damage reflected in DNA strand breaks under neutral or alkaline conditions. The neutral comet assay can detect both single-stranded and double-stranded DNA breaks and is able to identify DNA damage, which may be related to infertility [10]. The applications of the comet assay have increased significantly over the past few years as the sensitivity and accuracy of the technique has been recognized. Recently, we have modified the neutral comet assay to detect DNA strand breaks in boar spermatozoa during liquid storage [11, 13]. This approach includes a pre-treatment period of spermatozoa with 2% 2-mercaptoethanol to aid in membrane lysis and to help reduce the protamine disulphide bonds.
Our modified comet assay protocol also includes an extended period of enzymatic procedure with digestion buffers containing RNase A and HPLC-purified proteinase K (PK), without residual endonucleases [10]. Mercaptoethanol-treated sperm cells are basically sandwiched between thin layers of agarose on a glass microscope slide, lysed to remove proteins and lipids, treated with enzymes, electrophoresed, stained with a fluorescent dye and then the DNA remnants are visualized by fluorescence microscopy. The intensity of the stain in the comet tail region is presumed to be related to the DNA content, and DNA damage is estimated from measurements of the percent DNA in tail, tail length and tail moment, using an image analysis system (Komet Image Analysis System, UK). Spermatozoa with fragmented DNA (damaged) display increased migration of the DNA from the nucleus towards the anode, while spermatozoa with non-fragmented DNA (undamaged) do not form a “comet” [10, 11].

**DNA fragmentation in fish spermatozoa**

Salmonid fish are characterized by seasonal reproduction and spermatozoa are produced two months before spawning and then stored in the spermatic ducts [5]. Maintenance of genome integrity during the storage period is critical. Sperm DNA is vulnerable to oxidative damage that can result in DNA fragmentation. Therefore, testing DNA fragmentation is a prerequisite to evaluate sperm DNA status in salmonid fish, especially in relation to control of male reproduction in the aquaculture conditions.

**Comet assay of DNA fragmentation in fish spermatozoa**

Alkaline comet assay protocol, previously developed to detect DNA fragmentation in somatic cells, can be successfully applied for rainbow trout spermatozoa. The negative and positive (after exposure to 100 µM H$_2$O$_2$) controls were characterized by 15-30% and 60-80% tail DNA, respectively. In this protocol, hydrolysis with proteinase K, dithiothreitol or diiodosalicilate treatments are not required. Moreover, the application of these treatments in other protocols is most likely redundant [32].
Contrary to mammalian spermatozoa, fish spermatozoa are not resistant to the standard lysis conditions (presumably due to simplified structure) and thiol reagents are not needed to remove their nuclear proteins, indicating the lack of disulfide bonds in the nuclear proteins.

Screening of DNA fragmentation of six fish species revealed species-specific patterns. For most species, DNA fragmentation amounted to 18-28% and 42-83% of DNA in comet tail for negative and positive control, respectively (tab. 1). However, spermatozoa of zander and Siberian sturgeon were characterized by very high DNA fragmentation, which was evident in the negative control. As regards Siberian sturgeon spermatozoa, the high levels of DNA fragmentation may be species-specific because we did not observe this phenomenon for sperm DNA of lake sturgeon [6] or sterlet species (Ciereszko, unpublished).

<table>
<thead>
<tr>
<th>Species</th>
<th>Negative control</th>
<th>Positive control (+100 µM H₂O₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tail DNA (%)</td>
<td>Olive Tail Moment</td>
</tr>
<tr>
<td>Vimba</td>
<td>22.85 ± 6.22</td>
<td>4.91 ± 2.28</td>
</tr>
<tr>
<td>Siberian sturgeon</td>
<td>66.26 ± 4.59</td>
<td>24.06 ± 1.72</td>
</tr>
<tr>
<td>Carp</td>
<td>28.37 ± 3.71</td>
<td>7.92 ± 1.63</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>15.49 ± 0.58</td>
<td>4.14 ± 1.02</td>
</tr>
<tr>
<td>Zander</td>
<td>39.14 ± 6.59</td>
<td>11.79 ± 3.16</td>
</tr>
<tr>
<td>White fish</td>
<td>17.88 ± 0.65</td>
<td>3.03 ± 0.42</td>
</tr>
</tbody>
</table>

**Table 1. DNA fragmentation of fish spermatozoa (means±SEM)**

**Effects of cryopreservation on DNA integrity of boar spermatozoa**

The ultimate goal of this study was to show that supplementation of the freezing extender with lyophilized lipoprotein fractions extracted from ostrich egg yolk (LPFo) can give similar results, in terms of post-thaw sperm DNA damage as well as sperm quality, when compared with the freezing extender supplemented with whole hen yolk (HEY).
We have the modified neutral comet assay to detect DNA damage in cryopreserved boar spermatozoa [11]. Whole ejaculates or sperm-rich fractions of boar semen were frozen using a standard freezing protocol and were packaged in aluminium tubes or plastic straws [23]. An extender containing lactose-hen egg yolk with glycerol (lactose-HEY-G) or lactose, lyophilized lipoprotein fractions extracted from ostrich egg yolk and glycerol (lactose-LPFo-G) and Orvus Es Paste, respectively, was used. The sperm samples were also frozen in a standard boar semen extender (Kortowo-3, K-3; Olsztyn), without the addition of cryoprotective substances.

Sperm DNA damage was significantly increased following cryopreservation, irrespective of the extender type and packaging material [12]. The percentage of spermatozoa with damaged DNA was more attenuated in sperm samples frozen in K-3 extender, in the absence of cryoprotective substances, compared with lactose-HEY-G or lactose-LPFo-G extender. It should be stressed that throughout the entire experiments sperm DNA damage in samples frozen in lactose-LPFo-G extender was similar to those frozen in lactose-HEY-G extender. There were inter-boar variations in the degree of cryo-induced DNA damage. It seems that components of hen egg yolk or lyophilized lipoprotein fractions extracted from ostrich egg yolk afforded partial protection of DNA integrity of cryopreserved boar spermatozoa. Furthermore, the comets were differed markedly in percent tail comet and tail length, which suggest that the majority of variations in these parameters were due to inherent cell-cell differences within the sperm cells [14]. We found that the comet assay parameters, percent tail comet and tail length, were significantly increased in K-3 extender, indicating extensive sperm DNA damage [15]. Another observation from this study was that spermatozoa of the sperm-rich fraction were more vulnerable to cryo-induced DNA fragmentation than those of the whole ejaculate of boar semen [16].

The use of tritium-labelled actinomycin D (³H-AMD) showed that freezing-thawing caused disturbances in the chromatin organization of boar spermatozoa. This radioisotope method is based on the quantitative measurements of ³H-AMD, which has affinity for guanine-cytosine in the DNA molecule, incorporation into the sperm nuclei [24]. Increased
incorporation of $^3$H-AMD was more marked in sperm samples frozen in the absence of cryoprotective substances, indicating higher level of chromatin hypo-condensation (Fraser, unpublished). It was observed that there was a tendency for an increase in sperm chromatin instability in sperm-rich fraction compared with the whole ejaculate following freezing-thawing.

Accordingly, the deterioration in sperm DNA integrity was concomitant with a reduction in sperm motility, and loss of sperm plasma membrane integrity and mitochondrial activity following cryopreservation [12]. Fluorescent studies showed that post-thaw sperm plasma membrane integrity (SYBR-14-stained cells) and mitochondrial activity (Rhodamine 123) were significantly higher in sperm samples frozen in aluminium tubes compared with those frozen in straws [12]. We suggest that these differences might result from differential sensitivity to oxidative stress induced by the cryopreservation procedure.

It has been long recognized that the semen of certain individual boars survive freezing-thawing procedure better than that of others, and recent evidence suggests this may have a genetic basis [26]. Preliminary experiments support the hypothesis that consistent inter-individual variations in sperm freezability exist and may be genetically inherited. We have attempted to identify possible markers linked to genes controlling the ability of spermatozoa to freeze successfully, using DNA of individual boars. The DNA profile of each boar was analyzed using amplified restriction fragment length polymorphism (AFLP) technology and the individual boars were classified as "good" and "poor" freezers according to their post-thaw sperm quality. Preliminary data showed that the AFLP profile used to compare DNA genetic markers from the individual boars indicated gene differences, with post-thaw sperm quality, such as motility, plasma membrane integrity and mitochondrial function.

Fertility trials were conducted to assess the fertilizing capacity of boar spermatozoa frozen-thawed in lactose-HEY-G extender and lactose-LPFo-G extender. Fields trials were based on the artificial insemination of 10 weaned sows using sperm samples ($2 \times 10^9$ motile spermatozoa) packed in aluminum tubes. Two or three inseminations at 8-10-hours intervals were performed during a single estrus using a Soft & Quick® post-cervical catheter/cannula
set (Import-Vet, S.A, Spain). The non-return rate (30 days after insemination), which indicates the preliminary fertility results, was about 90%.

**DNA fragmentation in cryopreserved bull spermatozoa**

Cryopreservation of bull semen, in contrary to other farm animals, is commercially used in cattle breeding. Currently, the cryopreservation protocols ensure the maintenance of at least 50% post-thaw motile spermatozoa. Our results indicate that the sperm genome is also well preserved after cryopreservation. Using a modified protocol of the comet assay we found that the percentage of head DNA of fresh semen (81.6%) was not significantly decreased after cryopreservation (80.7%; n=12). However, a significant increase was found for Olive tail moment (4.70 and 5.41 for fresh and cryopreserved semen, respectively). It can be concluded that DNA fragmentation of cryopreserved bull semen is much lower than that of other farm animal species. However, since there were wide variations in sperm DNA fragmentation of individual bulls, ranging from 73 to 87%, further studies are needed to find out if these differences may be related to semen quality.

**Sperm DNA fragmentation in relation to liquid storage of turkey semen**

Short-term liquid storage of turkey semen is of a great interest in the management of turkey reproduction due to the extensive use of artificial insemination. Unfortunately, the duration of storage of undiluted or extended semen is very limited and a significant decrease in sperm viability and fertilizing capacity was recorded after 24 h of storage [9]. The comet assay used to analyze the nuclear DNA of turkey spermatozoa appears to be challenging due to difficulties in sperm lysis and DNA unwinding. A modified version of the comet assay includes treatments with proteinase K and dithiotheritol (to remove cross-linked proteins, such as protamines) and electrophoresis at low pH (due to abundance of alkali-labile sites in sperm DNA [22]. It should be noted that turkey spermatozoa are highly resistant to the lysis procedure when compared with those of other species
such as fish, bull and boar. It is possible that the very high resistance of nuclear DNA of turkey spermatozoa to the lysis procedure may be related to effective protection of DNA, which is necessary during prolonged storage in the female reproductive tract [3].

We analyzed DNA fragmentation (comet assay), motility characteristics (computer-assisted sperm analysis, CASA), and amidase activity (clinical assay) of turkey spermatozoa (n=9 to12) stored for 24 and 48 h (Ciereszko, unpublished). Even though there was an increase in amidase activity and sperm DNA fragmentation, there were no marked changes in sperm motility characteristics during liquid storage of turkey semen at 24 h. However, we found that a decrease in sperm motility characteristics was concurrent with an increase in amidase activity and DNA fragmentation during storage at 48 h. These data suggest that deterioration in turkey sperm quality during a short-term storage is mainly associated with sperm DNA damage and disruption of the sperm acrosome, resulting in premature activation of acrosomal serine proteinases. We observed that these changes were, to a lesser extent, accompanied by a decrease in sperm motility characteristics. Therefore, future studies should be focused on the development of extenders that can provide better protection of the functional integrity of turkey spermatozoa in order to increase the efficacy of liquid storage of turkey semen.

Use of the comet assay to study the effects of UV irradiation, oxidative stress and cryopreservation on DNA integrity of fish spermatozoa

Gynogenesis is based on artificially-induced parthenogenetic development followed by diploidization of the haploid zygotes to produce diploid offspring. Gynogenesis and gynogenetic fish have been used in experimental genetics and aquaculture, such as construction of genetic maps, studies of single locus effect and the sex determination mechanism, and rapid production of inbred lines or monosex populations [8]. Efficient induction of gynogenesis requires inactivation of sperm genome and high fertilization ability simultaneously. Therefore, the essential step of gynogenesis induction is to determine the optimal UV dose for inactivation of sperm genome.
In our study [7], we used the alkaline comet assay, CASA system and fertility experiments to evaluate DNA fragmentation, motility and fertilizing ability of rainbow trout spermatozoa following exposure to UV irradiation. Despite the dramatic increase in sperm DNA fragmentation there were no marked changes in sperm motility after exposure to UV irradiation for 5 min. We observed that prolonged exposure of spermatozoa to irradiation resulted in a decrease in motility characteristics and a gradual increase in DNA fragmentation. Furthermore, UV irradiation caused a significant decrease in the percentage of eyed embryos and most of the embryos did not hatch. Our results demonstrate that the alkaline comet assay can be used to monitor the effectiveness of fish sperm DNA inactivation by UV irradiation. We suggest that the comet assay, in conjunction with sperm motility analysis, can be applied for optimization of gynogenetic procedures in the fish.

We observed dual effects of oxidative stress on fish spermatozoa [7]. There was a marked increase in DNA fragmentation when highly diluted sperm suspensions (50 000-fold) were exposed to 0.1 mM $\text{H}_2\text{O}_2$. By contrast, when more concentrated sperm suspensions (diluted only 40-fold) were employed (to assess sperm motility and fertility at the same time) $\text{H}_2\text{O}_2$ used at concentrations ranging 1 to 20 mM caused only moderate increase in DNA fragmentation and a dose-dependent decline in sperm motility and fertilizing ability. These findings suggest that the toxic effects of $\text{H}_2\text{O}_2$ were mainly related to inhibition of motility of spermatozoa. However, the lack of effectiveness of $\text{H}_2\text{O}_2$ in inducing extensive DNA fragmentation is indicative of the presence of antioxidant defense mechanisms in rainbow trout spermatozoa.

HYDROLASES OF BOAR SEMINAL PLASMA – CATALYTIC PROPERTIES AND CODING SEQUENCES

Acid phosphatase

Electrophoretic studies on boar seminal plasma have shown that there are four molecular forms of acid phosphatase which, in different variants,
occur in the epididymal, prostatic and vesicular fluid. The activity of the
dominant epididymal forms is represented by about 90% of the total acid
phosphatase activity of the seminal plasma [28].

Protein complex of high-molecular weight with acid phosphatase
activity has been purified from the caudal epididymal fluid and whole
semen plasma of boar semen, using polyacrylamide gel electrophoresis
(PAGE) and electroelution. The protein complex isolated from the caudal
epididymal fluid consists of 3 fractions, with molecular weights more
than 60 kDa. On the other hand, the protein complex isolated from the
semen plasma is characterized by higher heterogeneity and consists of
fractions with molecular weights lower than 20 kDa, indicating that they
probably originate from the vesicular glands. It seems that changes in
the composition of high-molecular weight protein complex showing acid
phosphatase activity at ejaculation may affect its biological function.

We have isolated the molecular forms of epididymal acid phosphatases
from boar seminal plasma (EAcP) using dialysis and ion exchange
chromatography technique. The epididymal fraction obtained by
chromatography on DEAE Sepharose FF column contained only one
molecular form of epididymal acid phosphatases, which exhibited the highest
electrophoretic mobility (mEAcP). The applied isolation procedure gave
more than 7000-fold enzyme purification and the yield efficiency was about
50% [31]. Some selected biochemical and catalytic properties of the isolated
molecular form of mEAcP and the vesicular acid phosphatase (mVAcP),
isolated from the boar vesicular fluid [29] are presented in Table 2.

There was immunological similarity between the epididymal and
vesicular forms of acid phosphatase. The use of polyclonal antibody against
mEAcP immobilized on affinity gel enabled efficient isolation of both
epididymal and vesicular forms of acid phosphatase from boar seminal
plasma. Acid phosphatases, with molecular weights approximately 45 kDa
or 50 kDa (pI 7.0), have been detected in the boar seminal plasma, using
two-dimensional (2D) electrophoresis and immunoblotting [30]. It should
be noted that the activity of acid phosphatases in the male reproductive
tract has been found to be species-specific. In the case of the boar, the
exceptionally high activity of acid and alkaline phosphatases may due to
intensive biochemical processes associated with maturation of a very large number of spermatozoa.

Since many acid phosphatases exhibit activity towards phosphorylated protein substrates, their action may have a considerable effect on the biological properties of spermatozoa. In a previous study it was reported that the electrophoretic heterogeneity of acid phosphatase of boar epididymis might be related to post-translational adaptation to specific biochemical processes accompanying sperm maturation in different segments of the epididymis [28]. We suggest that the molecular form of mEAcP may play an essential role in these processes.

Interestingly, the partial inhibition of mEAcP by sodium orthovanadate, ammonium molybdate and tartaric acid differentiates this phosphatase from other acid phosphatases originating from the seminal vesicle fluid. The results of a study reported by Strzeżek et al [25] showed that the gradual disappearance of the molecular forms of epididymal acid phosphatase, accompanied by increased activity of the vesicular molecular form during administration of a synthetic antiandrogen (cyproterone acetate), indicates that an androgen-dependent mechanism is involved in the control

<table>
<thead>
<tr>
<th>Property</th>
<th>Vesicular acid phosphatase (mVAcP)</th>
<th>Epididymal acid phosphatase (mEAcP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>42 kDa</td>
<td>50 kDa</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>7.1</td>
<td>7.0</td>
</tr>
<tr>
<td>Optimum pH</td>
<td>5.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Thermostability</td>
<td>70°C</td>
<td>70°C</td>
</tr>
<tr>
<td>$K_m$ values for substrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-nitrophenyl phosphate</td>
<td>$0.87 \times 10^{-3}$ M</td>
<td>$0.63 \times 10^{-3}$ M</td>
</tr>
<tr>
<td>phenylphosphate</td>
<td>$1.62 \times 10^{-3}$ M</td>
<td>$1.5 \times 10^{-3}$ M</td>
</tr>
<tr>
<td>phosphotyrosine</td>
<td>$0.37 \times 10^{-3}$ M</td>
<td>$2.1 \times 10^{-3}$ M</td>
</tr>
<tr>
<td>Percent inhibition (%) with 5 mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tartaric acid</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>sodium orthovanadate</td>
<td>100</td>
<td>35</td>
</tr>
<tr>
<td>ammonium molybdate</td>
<td>100</td>
<td>70</td>
</tr>
</tbody>
</table>

Wysocki P, Strzeżek J [29, 31]
of expression of genes encoding acid phosphatases in boar epididymis. Moreover, mEAcP seems to have different functions compared with the molecular form of acid phosphatases previously isolated from the fluid of boar vesicular glands [29].

It should be noted that the epididymal acid phosphatase accompanies spermatozoa during the maturation process while spermatozoa come into contact with the vesicular acid phosphatase only after ejaculation. Evidence has been shown that the vesicular acid phosphatase exhibited lower affinity for phosphotyrosine compared with the mEAcP, and was inhibited only by about 35% by 5 mM sodium orthovanadate, a tyrosine phosphatase inhibitor [31]. Furthermore, mEAcP does not also exhibit the kinetic properties of high-molecular weight acid phosphatases (>100 kDa), which are inhibited by micromolar concentrations of tartaric acid [27]. It was observed that 5 mM tartaric acid inhibited mEAcP activity by 50%. These findings indicate that the purified epididymal molecular form of acid phosphatase does not fulfill the entire catalytic criteria of phosphotyrosine phosphatases, for example, it was not fully inhibited by 0.1 to 1 mM sodium orthovanadate. Furthermore, these results indicate that mEAcP is an enzyme with low substrate specificity and has a wide pH range compared with the vesicular acid phosphatase. The distinct properties of mEAcP were not shown by acid phosphatase purified from the vesicular fluid, indicating that mEAcP may have physiological role in boar epididymis.

Attempts have been made to analyze the encoding nucleotide sequence for gene of acid phosphatase isolated from boar vesicular glands [28]. The degenerated primers used were based on the N-terminal amino acid sequence of the enzyme. Numerous amplifications of the fragments were obtained, when using these primers. New primers were developed on the basis of the nucleotide sequence of 111 base pair fragment for the gene of boar acid phosphatase, ACP (GeneBank, accession No. AF 222911). Our preliminary studies showed that the encoding gene sequence for acid phosphate isolated from the fluid of the vesicular glands has 330 base pair fragment, which shows strong sequence homology with that of human, mice and rats (Wysocki, unpublished).
PAF-AH

The procedure used for isolation of PAF-AH from boar seminal plasma was described in a previous study [19]. The obtained protein samples were subjected to sulphydryl alkylation with iodoacetamide and then chemically deglycosylated with trifluoromethanesulfonic acid (TFMS). The isolation procedure enabled the purification and further analysis of the N-terminal sequence of the lowest molecular weight polypeptide (43 kDa), which is a component of the protein complex of PAF-AH of boar seminal plasma. This sequence is not homologous with the characteristic sequences of PAF-AH isolated from bull seminal plasma [18], as well as the sequences of the enzyme isolated from other sources (according to BLAST database). However, the analyzed protein fraction is homologous with the characteristic sequences of IgG-binding proteins and zona pellucida-binding adhesion proteins, zonadhensions [20]. Evidence has been shown that PAF-AH binds strongly to the plasmalemma of boar spermatozoa [19]. It can be suggested that the 43 kDa protein component of PAF-AH complex facilitates its adhesion properties.

The use of cyanogen bromide for chemical cleavage of polypeptides, with molecular weights of 100 kDa (PAF-AH 1) and 65 kDa (PAF-AH 2), yielded two fractions with 30 kDa and 43 kDa, respectively. Using 2D-electrophoresis, western blotting and immunodetection technique, we have detected complexes with PAF-AH activity in boar seminal plasma by polypeptide mapping. A polypeptide with a molecular weight of 100 kDa has an isoelectric point (pI) of 3.5, whereas polypeptides with lower molecular weights have a pI of about 8.0 (Wysocki, unpublished).

REFERENCES

1. Alvarez JG, Ollero M, Larson-Cook KL, Evenson DP 2004 Selecting cryopreserved semen for assisted reproductive techniques based on the level of sperm nuclear DNA fragmentation resulted in pregnancy. Fertility and Sterility 81 712-713.


Biochemical markers of animal semen


