Animal Science Papers and Reports vol. 32 (2014) no. 4, 327-336
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

The ability of zinc-binding proteins from boar seminal plasma to bind heparin and phosphorylcholine*

Marzena Mogielnicka-Brzozowska, Jerzy Strzeżek, Anna Dziekońska, Władysław Kordan**

University of Warmia and Mazury in Olsztyn, Faculty of Animal Bioengineering,
Department of Animal Biochemistry and Biotechnology,
Oczapowskiego 5, 10-718 Olsztyn, Poland

(Accepted October 30, 2014)

Zinc-binding proteins (ZnBPs) isolated from boar seminal plasma exert multiple effects on boar spermatozoa. The aim of this study was to determine whether the isolated zinc-binding proteins were capable of binding additional ligands. The affinity chromatography method was used for the first time to demonstrate that boar ZnBPs show affinity for both heparin (hep) and phosphorylcholine (pch). ZnBPs$^+$hep accounted for approximately 20%, and ZnBPs$^+$pch – for approximately 45% of total ZnBPs. Predominant polypeptides with molecular mass form 6.5 to 14 kDa were observed in both groups (ZnBPs$^+$hep, ZnBPs$^+$pch). The analyzed peptides' low molecular mass and their ability to bind zinc ions as well as heparin and phosphorylcholine suggest that they belong to the family of multifunctional boar spermadhesins which perform their functions in the fertilization process by binding more than one ligand. The results indicate that the ZnBPs of boar seminal plasma may be involved in the processes associated with oocyte fertilization not only by zinc ions binding but also through heparin and phosphorylcholine.

KEY WORDS: boar seminal plasma / heparin-binding protein / phosphorylcholine-binding protein / zinc-binding proteins

Seminal plasma contains substances required for sperm maturation and egg fertilization. Functions of seminal plasma proteins are inextricably linked to the ability

*This study was supported by funds from the University of Warmia and Mazury in Olsztyn (No. 0103.0803) and KBN grant No. 2 PO6D 001 28.
**Corresponding author: mmog@uwm.edu.pl

of binding a variety of ligands. Zinc-binding proteins play various roles at different stages of fertilization. They regulate sperm chromatin condensation, influence sperm motility and exert antioxidant, antibacterial and immunosuppressive effects [Silvestroni *et al.* 1989, Assreuy *et al.* 2003]. In addition to the ZnBPs isolated in our study, the most extensively researched groups of boar seminal plasma proteins include peptides with molecular mass of 25, 38 and 64 kDa [Strzeżek and Hopfer 1987], spermadhesins PSPI/PSP-II [Campanero-Rhodes *et al.* 2005] and 54kDa glycoprotein [Hołody and Strzeżek 1999, Płucienniczak *et al.* 1999]. ZnBPs in human seminal plasma include semenogelins I and II and the prostate-specific antigen (PSA) [Mogielnicka-Brzozowska and Kordan 2011]. ZnBPs have also been identified in the seminal plasma of dog [Mogielnicka-Brzozowska *et al.* 2012]. The characteristic properties of ZnBPs can be attributed to their ability to bind zinc ions as well as additional ligands. The binding of phospholipids containing phosphorylcholine residues is responsible for sperm coating with seminal plasma proteins upon ejaculation, and the above was observed in low-molecular-weight proteins in bull – PDC-109, BSP-A1, BSP-A2, BSP-A3 and BSP 30kDa [Leblond *et al.* 1993, Desnoyers and Manjunath 1992] according to the new nomenclature called Binder of Sperm [Manjunath *et al*. 2008]. BSP homologues have been isolated from the seminal plasma of human, boar, hamster, mouse and rat [Leblond *et al.* 1993]. Seminal plasma proteins secreted by seminal vesicles bind to the choline-containing phospholipids of the sperm plasma membrane during ejaculation and induce the secretion of cholesterol, which initiates the capacitation process [Therien *et al.* 1999, Manjunath and Therien 2002, Swamy 2004].

Seminal plasma proteins are capable of binding both choline-containing phospholipids and heparin. Proteins of that type have been identified in the seminal plasma of bull, boar and human [Calvete *et al.* 1995, Romero *et al.* 1997, Jonakova and Ticha 2004]. Heparin-binding proteins isolated from human seminal plasma and analysed by mass spectrometry revealed numerous proteins capable of binding zinc ions, including semenogelins I and II, albumins, zinc fingers and transcription factors [Kumar *et al.* 2009].

The aim of this study was to determine the ability of zinc-binding proteins of boar seminal plasma to bind additional ligands, including heparin and phosphorylcholine, that would allow a better understanding of the mechanisms of their effects on fertilization process.

Material and methods

Animals and semen collection

Semen was collected once a week from 3 Polish Large White boars aged 2 years, once a week, over a period of 3 weeks. The boars were fed a commercial pig ration. Water was available *ad libitum*. Semen was collected with the gloved-hand technique, and the gel portion removed with a nylon mesh filter. Ejaculates were centrifuged at 700 x G, (5 min at room temperature) to separate seminal plasma and spermatozoa. The

supernatant was centrifuged 10 000 x G, 15 min at room temperature). The resulting seminal plasma was stored at -20°C for further analysis. The experimental protocol was approved by the Local Ethics Committee for Animal Experimentation.

Protein content analysis

Total protein content was measured according to Lowry *et al*. [1951] with the use of bovine serum albumin (BSA, Serum and Vaccine Production, Cracow, Poland) as the standard.

Isolation of zinc-binding proteins

Before chromatographic separation, seminal plasma was dialyzed for 24 h against deionised water and centrifuged (10 000 x G, 15 min at room temperature). The supernatant was used to isolate zinc-binding proteins (ZnBPs) by the method described by Mogielnicka-Brzozowska *et al.* [2011]. The resulting ZnBP fractions were dialysed against deionised water and stored at -80°C.

Isolation of heparin-binding ZnBPs

Heparin-binding proteins were isolated in line with the technique described by Calvete *et al.* [1997] with the use of the FPLC (AMERSHAM BIOSCIENCES) system and the affinity chromatography method in the Heparin Sepharose CL 6B medium, XK 16 column (AMERSHAM BIOSCIENCES), at a flow rate of 1 ml/min. The affinity medium was equilibrated and samples were applied with the use of an equilibration buffer composed of 0.02 M sodium phosphate and 0.15 M NaCl, pH 7.3. 5 ml of the solution containing isolated ZnBPs (protein concentrations 10 mg/ml) was applied to the medium. Non heparin-binding ZnBPs ($ZnBPs^{-}$hep) were eluted from the medium with the equilibration buffer. Heparin-binding ZnBPs ($ZnBPs^{+}$hep) were rinsed with 3M NaCl solution. Fractions with the volume of 2 ml were collected and dialysed against deionised water for 24 hours at 4°C. They were condensed in dialysis sacks with polyethylene glycol at 4°C and stored at -80°C for further analysis.

Isolation of phosphorylcholine-binding ZnBPs

Phosphorylcholine-binding ZnBPs were isolated by the affinity chromatography method in the Immobilized p-Aminophenyl Phosphoryl Choline Agarose (PIERCE) medium. Chromatographic separation was performed in line with the procedure described by Calvete *et al.* [1997] in the FPLC system (AMERSHAM BIOSCIENCES). The solution of isolated ZnBPs (3 ml, protein concentrations of 10 mg/ml) was applied to the medium and incubated at 4°C for 24 hours. Fractions with the volume of 2 ml were collected. The samples were desalted in the Sephadex G 25 Medium (AMERSHAM BIOSCIENCES), and deionised water was used as eluent. The fractions were condensed in dialysis sacks with polyethylene glycol at 4°C. They were stored at -80°C for further analysis.

Electrophoretic properties of isolated fractions in the bis-Tris-SDS-PAGE system

Gels were prepared according to the procedure recommended by INVITROGEN. Proteins were separated in 12% polyacrylamide gel and 8% stacking gel with 0.375 M bis-Tris-HCl (pH 6.8) gel buffer. Electrophoresis was carried out in buffer containing: 0.05 M MES (2-(N-morpholino) ethanesulfonic acid), 0.05 M Tris; 0.001 M EDTA; 0.1 % SDS (w/v); 0.001 M sodium dithionate, at 130 V. Proteins of decreasing concentrations from 40μg do 10μg were dispensed into wells in the gel material to determine a broad range of molecular mass values of proteins from each fraction. The separated protein fractions were silver-stained with the Silver Staining Kit (AMERSHAM BIOSCIENCES) according to the method proposed by Heukeshoven and Dernick [1985]. Wide Range Standards (SIGMA) was applied as the molecular mass standard. Electropherograms were analysed in the MultiAnalyst version 1.1 (BioRad) application.

Results and discussion

Zinc-binding proteins were isolated from boar seminal plasma and characterised in our previous study [Mogielnicka-Brzozowska *et al.* 2011]. In their native state they possess antigenic character and form high-molecular-weight aggregates [Mogielnicka-Brzozowska *et al.* 2013]. They exert protective effects on the sperm plasma membrane and enhance the motility of boar spermatozoa stored in a liquid state at 4°C [Mogielnicka-Brzozowska *et al.* 2011]. Seminal plasma proteins perform their functions in the fertilization process by binding more than one ligand [Jonakova and Ticha 2003, Mogielnicka-Brzozowska and Kordan 2011].

ZnBPs were separated into two fractions by affinity chromatography in a heparin-containing medium (Fig. 1). Fraction (A) contained non heparin-binding ZnBPs ($ZnBPs^{-}hep$), and fraction (B) – heparin-binding ZnBPs ($ZnBPs^{+}hep$). A planimetric analysis revealed that $ZnBPs^{+}hep$ accounted for approximately 20% of ZnBPs applied to the column.

The electropherograms of the $ZnBPs^{-}hep$ fraction revealed a group of predominant peptides with molecular mass of <6.5 to 30 kDa and a 53 kDa protein (Fig. 2A line 1). Five polypeptides with molecular mass of <6.5, 6.5, 11, 14 and 16 kDa were observed when a smaller amount of protein was applied to the gel material (Fig. 2A line 4). The $ZnBPs^{+}hep$ fraction consisted of a group of predominant polypeptides with molecular mass of <6.5 to 25 kDa and a single 90 kDa band (Fig. 2B line 1). Five low-molecular-weight polypeptides with molecular mass of <6.5, 6.5, 11, 14 and 16 kDa were revealed when a reduced amount of protein was dispensed on the gel (Fig. 2B line 4).

Present findings suggest that approximately 20% of boar ZnBPs are capable of binding a second ligand – heparin. Heparin- and chondroitin sulfate-like glycosaminoglycans (GAGs) are secreted by female reproductive tract epithelial cells, in particular during the follicular phase of the estrous cycle. GAGs play an

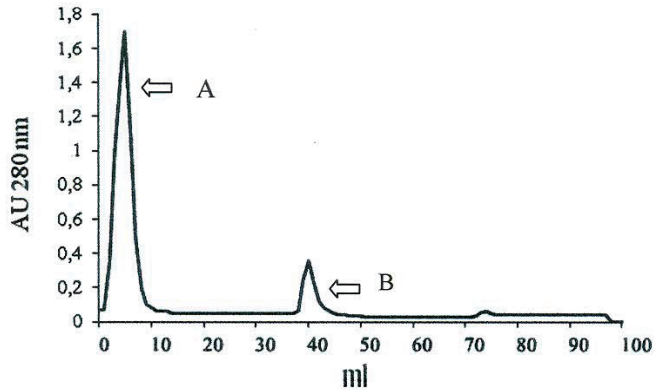

Fig. 1. Graphic image plotted by UNICORN Control System software (AMERSHAM BIOSCIENCES) of (A) zinc-binding non heparin-binding ($ZnBPs^{-}$hep) and (B) heparin-binding ($ZnBPs^{+}$hep) of boar seminal plasma separated on Heparin Sepharose CL6B gel (AMERSHAM BIOSCIENCES).

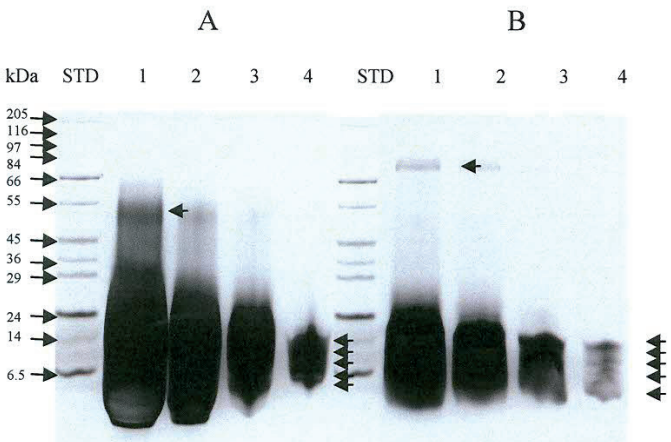

Fig. 2. Sodium dodecyl sulphate polyacryloamide gel electrophoresis (SDS PAGE) of isolated (A) zinc-binding non heparin-binding ($ZnBPs^{-}$hep) and (B) heparin-binding ($ZnBPs^{+}$hep) of boar seminal plasma. Different amounts of protein were loaded into the gel wells to obtain optimal resolution of the protein bands. Lane 1:40 µg. Lane 2: 30 µg. Lane 3: 20 µg. Line 4: 10 µg. STD – Wide Range Standards (SIGMA).

important role in the sperm cells transformation that occur before fertilization. The content of GAGs on the sperm plasma membrane changes during the transport of sperm inside the female reproductive tract. The dissociation of GAGs from the surface of the sperm plasma membrane probably induces the heparin-dependent increase in Ca^{2+} concentrations in the acrosomal matrix, which initiates capacitation [Florman and First 1988]. The ability of ZnBPs to bind heparin suggests that those proteins participate in the regulation of capacitation processes and acrosome reactions in pigs.

ZnBPs were separated into two fractions by affinity chromatography in a phosphorylcholine-containing medium (Fig. 3). Fraction (C) containing zinc-binding proteins which did not bind phosphorylcholine (ZnBPs$^-$pch), and fraction (D) containing ZnBPs which showed affinity for phosphorylcholine (ZnBPs$^+$pch). A planimetric analysis revealed that ZnBPs$^+$pch accounted for approximately 45% of ZnBPs applied to the column.

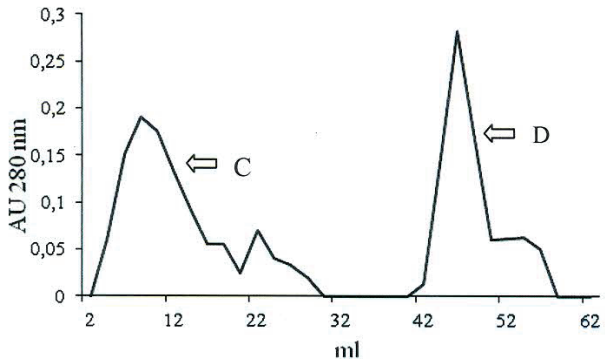

Fig. 3. Graphic image plotted by UNICORN Control System software (Amersham Biosciences) of (C) zinc-binding non phosphorylcholine-binding (ZnBPs$^-$pch) and (D) phosphorylcholine-binding (ZnBPs$^+$pch) of boar seminal plasma separated on Immobilised p-Aminophenyl Phosphoryl Choline Agarose (PIERCE).

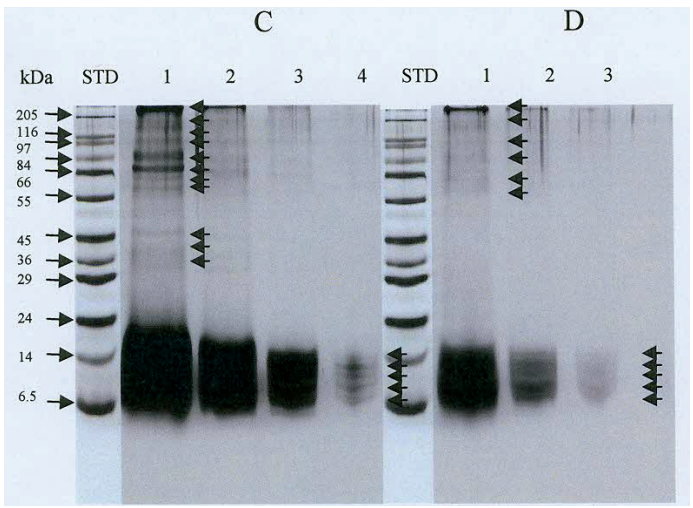

Fig. 4. Sodium dodecyl sulphate polyacryloamide gel electrophoresis (SDS PAGE) of isolated (C) zinc-binding non phosphorylcholine-binding (ZnBPs$^-$pch) and (D) phosphorylcholine-binding (ZnBPs$^+$pch) of boar seminal plasma. Different amounts of protein were loaded into the gel wells to obtain optimal resolution of the protein bands. Lane 1:40µg. Lane 2: 30µg. Lane 3: 20µg. Line 4: 10 µg. STD – Wide Range Standards (SIGMA).

ZnBPs$^-$pch electropherograms revealed the presence of predominant low-molecular-weight polypeptides with molecular mass of <6.5 to 20 kDa and 30, 40, 45, 60, 63, 66, 84, 97, 130 and >205 kDa (Fig. 4C line 1). Five polypeptides with molecular weight of 6.5, 8, 10, 12 and 14 kDa were observed when lower protein concentrations were applied to the gel (Fig. 4C line 4). The ZnBPs$^+$pch fraction was represented by a group of predominant polypeptides with molecular weight of 6.5 to 14 kDa and 6 bands with molecular mass of 60, 63, 84, 97, 130 and >205 kDa (Fig. 4D line 1). The application of smaller amounts of protein produced five fractions with molecular weight of 6.5, 8, 10, 12 and 14 kDa (Fig. 4D line 4).

ZnBPs bind with the phosphorylcholine residues of plasma membrane lipids to coat sperm cells. Phosphorylcholine binding regulates the aggregation of seminal plasma proteins [Gasset *et al.* 1997] and contributes to the maturation of epididymal sperm [Tahnert *et al.* 2007, Sahin *et al.* 2009]. Phosphorylcholine-binding proteins also participate in the formation of sperm reserves in female oviducts [Gwathemey *et al.* 2006].

This is the first ever study to demonstrate that nearly one-half (45%) of boar ZnBPs bind phosphorylcholine. Phosphorylcholine-containing phospholipids account for more than 60% of all phospholipids in the boar sperm plasma membrane [Parks *et al.* 1987]. The binding of phosphorylcholine-containing phospholipids may be the main mechanism responsible for sperm coating with ZnBPs which protects sperm cells against cold shock damage observed at our previous study [Mogielnicka-Brzozowska *et al.* 2011].

Electrophoretic images of the non-binding and binding protein groups (ZnBPs$^-$hep/ZnBPs$^+$hep and ZnBPs$^-$pch/ZnBPs$^+$pch) were similar. Polypeptides resulting from the action of denaturing and reducing agents on the native structure of these non-binding and binding proteins have similar molecular weights. Closer identification of polypeptides specific to particular groups of proteins requires further detailed study.

Interestingly, polypeptides with the same low molecular weight (from 6.5 to 14 kDa) were reported in both groups (ZnBPs$^+$pch, ZnBPs$^+$hep), which could indicate that some polypeptides are capable of binding all three ligands (zinc ions, heparin and phosphorylcholine). This possibility was also suggested by Jonakova and Ticha [2003] who demonstrated that low-molecular-weight boar spermadhesins bind several types of ligands, including heparin, phosphorylcholine, mannose and the *zona pellucida*. Spermadhesins are low-molecular-weight (12-16 kDa) glycoproteins whose biological activity is determined by the glycosylation state, aggregation and ligand binding ability. The discussed group of glycoproteins comprises AQN-1, AQN-2, AQN-3, AWN-1, AWN-2, PSP-I and PSP-II [Rodriguez-Martinez *et al.* 2011]. According to Calvete *et al.* [1997], spermadhesins account for around 90% of all seminal plasma proteins in boar. The two main spermadhesins, PSP-I and PSP-II, represent more than 50% of all seminal plasma proteins in the discussed animal species [Rutherfurd *et al.* 1992]. Spermadhesins are produced mainly by the accessory glands of the male reproduction system, and they become mixed with sperm cells

during ejaculation. Spermadhesins coat the surface and stabilize the sperm plasma membrane, they participate in capacitation and sperm-oocyte interactions [Sanz *et al.* 2003, Rodriguez-Martinez *et al.* 2011]. Hołody and Strzeżek [1999] demonstrated that boar spermadhesins are capable of binding both heparin and zinc ions.

The analysed polypeptides' low molecular weight (from 6.5 to 14 kDa) and their ability to bind zinc ions as well as heparin and phosphorylcholine suggest that they belong to the family of multifunctional boar spermadhesins which perform their functions in the fertilization process by binding various ligands at different stages of sperm maturation and during sperm-oocyte interactions.

Our results indicate that zinc-binding proteins from boar seminal plasma play an essential role in the processes associated with egg fertilization, in particular by interacting with phosphorylcholine residues of sperm plasmalema and by heparin binding inside the female reproductive tract. However, further work is needed to expand our knowledge of molecular mechanisms that underlie the discussed processes.

REFERENCES

1. ASSREUY A.M., ALENCAR N.M.N., CAVADA B.S., ROCHA-FILHO D.R., FEITOSA R.F.G., CUNHA F.Q., CALVETE J.J., RIBEIRO R.A., 2003 – Porcine Spermadhesins PSP-I/PSP-II stimulates macrophages to realease a neutrophil chemotactic substance: modulation by mast cells. ***Biology of Reproduction*** 68,1836-1841.
2. CALVETE J.J., RAIDA M., GENTZEL M., URBANKE C., SANZ L., TÖPFER -PETERSEN E., 1997 – Isolation and characterization of heparin and phosphorylcholine-binding proteins of boar and stallion seminal plasma. Primary structure of porcine pB1. ***FEBS Letters*** 407, 201-206.
3. CALVETE J.J., SANZ L., REINERT M., TÖPFER-PETERSEN E., 1995 – Heparin-binding proteins on bull, boar, stallion, and human spermatozoa. In: Jamieson B.G.M., Ausio J., Justine J. L. Advances in Spermatozoal Phylogeny and Taxonomy. ***Memoires du Museum Nantional d' Histoire Naturelle*** 166, 515-524.
4. CAMPANERO-RHODES M.A., MENENDEZ M., SAINZ L., CALVETE J.J., SOLIS D., 2005 – Analysis of the stability of the spermadhesin PSP-I/ PSP-II heterodimer. Effects of Zn^{2+} and acidic pH. ***FEBS Journal*** 272**,** 5663–5670.
5. DESNOYERS L., MANJUNATH P., 1992 – Major proteins of bovine seminal plasma exhibit novel interactions with phospholipid. ***Biological Chemistry*** 267, 10149-10155.
6. FLORMAN H.M., FIRST N.L., 1988 – The regulation of acrosomal exocytosis. I. Sperm capacitation is required for the induction of acrosome reaction by the bovine zona pellucida in vitro. ***Developmental Biology*** 128, 453-463.
7. GASSET M., SAIZ J.L., LAYNEZ J., SANZ L., GENTZEL M., TOPFER-PETERSEN E., CALVETE J.J.,1997 – Conformational features and thermal stability of bovine seminal plasma protein PDC-109 oligomers and phosphorylcholine – bound complexes. ***European Journal of Biochemistry*** 250, 735-744.
8. GWATHMEY T.M., IGNOTZ G.G., MUELLER J.L., MANJUNATH P., SUAREZ S.S.,2006 – Bovine seminal plasma proteins PDC-109, BSP-A3, and BSP-30-kDa share functional roles in storing sperm in the oviduct. ***Biology of Reproduction*** 75, 501-7.
9. HEUKESHOVEN J., DERNICK R., 1985 – Simplifled method for silver staining of proteins in polyacrylamide gels and the mechanism of silver staining. ***Electrophoresis*** 6, 103-112.
10. HOLODY D., STRZEZEK J. 1999 – Heparin- and Zn^{2+}-binding proteins from boar seminal plasma. ***Acta Biochimica Polonica*** 46**,** 935-939.

11. JONAKOVA V., TICHA M. 2003 – Boar seminal plasma proteins and their binding properties. A review. ***Collection of Czechoslovak Chemical Communications*** 69, 461-475.
12. KUMAR V., HASSAN M., TOMAR A.K., KASHAV T., NAUTIYAL J., SINGH S., SINGH T., YADAV S., 2009 – Proteomic analysis of heparin-binding proteins from human seminal plasma: a step towards identification of molecular markers of male fertility. ***Journal of Biosciences*** 34, 899-908.
13. LEBLOND E., DESNOYERS L., MANJUNATH P., 1993 – Phosphorylcholine-binding proteins from the seminal fluids of different species share antigenic determinants with the major proteins of bovine seminal plasma. ***Molecular Reproduction and Development*** 34, 443-449.
14. LOWRY I.O.M., ROSENBROUGH N., FARR R.L., RANDALL R.J., 1951 – Protein measurement with the folinphenol reagent. ***Journal of Biological Chemistry*** 193, 265-275.
15. MANJUNATH P., THERIEN I., 2002 – Role of seminal plasma phospholipid-binding proteins in sperm membrane lipid modification that occurs during capacitation. ***Journal of Reproductive Immunology*** 53, 109-119.
16. MANJUNATH P., LEFEBVRE J., JOIS P.S., FAN J., WRIGHT W.W., 2008 – New nomenclarure for mammalian BSP genes. ***Biology of Reproduction*** 80, 394-397.
17. MOGIELNICKA-BRZOZOWSKA M., FRASER L., CZARZASTA J., KORDAN W., 2012 – Isolation and characterization of zinc-binding proteins from canine seminal plasma. ***Polish Journal of Veterinary Sciences*** 15, 493-498.
18. MOGIELNICKA-BRZOZOWSKA M., FRASER L., STRZEŻEK J., KORDAN W., 2013 – The antigenic character of zinc-binding proteins and their localization in boar reproductive tract – A preliminary study. ***Acta Biochimica Polonica*** 60, 255-258.
19. MOGIELNICKA-BRZOZOWSKA M., KORDAN W., 2011 – Characteristics of selected seminal plasma proteins and their application in the improvement of the reproductive processes in mammals. ***Polish Journal of Veterinary Sciences*** 14, 489-499.
20. MOGIELNICKA-BRZOZOWSKA M., WYSOCKI P., STRZEZEK J., KORDAN W., 2011 - Zinc–binding proteins from boar seminal plasma - isolation, biochemical characteristics and influence on spermatozoa stored at 4°C. ***Acta Biochimica Polonica*** 58, 171-177.
21. PARKS J.E., ARION J.W., FOOTE R.H., 1987 – Lipids of plasma membrane and outer acrosomal membrane from bovine spermatozoa. ***Biology of Reproduction*** 37, 1249-1258.
22. PLUCIENNICZAK G., JAGIELLO A., PLUCIENNICZAK A., HOLODY D., STRZEZEK J., **1999 -** Cloning of complementary DNA encoding the pB1 component of the 54-kDa glycoprotein of boar seminal plasma. ***Molecular Reproduction and Development*** 52, 303-309.
23. RODRIGUEZ-MARTINEZ H., KVIST U., ERNERUDH J., SANZ L., CALVETE J.J., 2011 – Seminal plasma proteins: What role do they play? ***American Journal of Reproductive Immunology*** 66, 11-22.
24. ROMERO A., VARELA P.F., TÖPFER-PETERSEN E., CALVETE J.J., 1997 – Crystallization and preliminary X-ray diffraction analysis of bovine seminal plasma PDC-109, a protein composed of two fibronectin type II domains. ***Proteins*** 28, 454-456.
25. RUTHERFURD K.J., SWIDEREK M.M., GREEN C.B., CHEN S., SHIVELY J.E., KWOK S.C.M., 1992 – Purification and characterization of PSP-I and PSP-II, two major proteins from porcine seminal plasma. ***Archives of Biocheistry and Biophysics*** 295, 352-359.
26. SAHIN E., PETRUNKINA A., EKHLASI-HUNDRIESER M., HETTEL C., WABERSKI D., HARRISON R., TÖPFER-PETERSEN E., 2009 – Fibronectin type II-module proteins in the bovine genital tract and their putative role in cell volume control during sperm maturation. ***Reproduction and Fertility Developoment*** 21, 479-88.
27. SANZ L., CALVETE J., MANN K., GABIUS H., TÖPFER-PETERSEN E., 1993 – Isolation and biochemical characterisation of heparin-binding proteins from boar seminal plasma: a dual role for spermadhesines in fertilization. ***Molecular Reproduction and Development*** 36, 37-43.

28. SILVERSTRONI L., MENDITTO A., MODESTI A., SCARPA S., 1989 – Zinc uptake in human seminal spermatozoa: characterization and effects on cell membranes. ***Archives of Andrology*** 23, 97-103.
29. STRZEŻEK J., HOPFER E., 1987 – Zinc ion-dependent protein in boar semen. I. Egg yolk precipitating activity and some biochemical proteins. ***Animal Reproduction Science*** 13, 117-131.
30. SWAMY M.J. 2004 – Interaction of bovine seminal plasma proteins with model membranes and sperm plasma membranes. ***Current Science*** 87, 203-211.
31. TANNERT A., KURZ A., ERLEMANN K.R., MÜLLER K., HERRMANN A., SCHILLER J., TÖPFER-PETERSEN E., MANJUNATH P., MÜLLER P. 2007 – The bovine seminal plasma protein PDC-109 extracts phosphorylcholine-containing lipids from the outer membrane leaflet. ***European Biophysics Journal*** 36, 461-475.
32. THÉRIEN I., MOREAU R., MANJUNATH P. 1999 – Bovine seminal plasma phospholipid-binding proteins stimulate phospholipid efflux from epididymal sperm. ***Biology of Reproduction*** 61, 590-598.