RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.2.425-431

Isolation and characterisation of binder of sperm proteins in Vechur bull seminal plasma

Sophia Xavier[1*], Hiron M. Harshan[2], C. Jayakumar[3], Leeba Chacko[4], M. Shynu[5] and Elizabeth Kurian[6]
Department of Animal Reproduction, Gynaecology and Obstetrics
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651
Kerala Veterinary and Animal Sciences University
Kerala, India

Citation: Xavier, S., Harshan, H.M., Jayakumar, C., Chacko, L., Shynu, M. and Kurien, E. 2024. Isolation and characterisation of binder of sperm proteins in Vechur bull seminal plasma.
J. Vet. Anim. Sci. **55**(2):425-431
DOI: https://doi.org/10.51966/jvas.2024.55.2.425-431

Received: 13.02.2024 *Accepted: 20.03.2024* *Published: 30.06.2024*

Abstract

Binder of sperm (BSP) proteins represent a major class of proteins present in seminal plasma of ungulates. These proteins play a crucial role in various stages of fertilisation. The proteins have been extensively studied in purebred exotic cattle and many other species of ungulates. However, limited studies have been conducted with respect to BSP proteins in Vechur bull. This study was directed at isolation and characterisation of BSP proteins in Vechur bull seminal plasma. Seminal plasma was harvested from 36 ejaculates obtained from six healthy adult Vechur bulls. The mean protein content of seminal plasma was determined to be 84.10 ± 2.73 mg/mL. The proportion of BSP proteins recovered by gelatin affinity chromatography was 37.89 ± 0.83 per cent of the total protein. The SDS-page profiling of seminal plasma proteins revealed 20 protein bands in the 10-180 kDa region. Three bands were observed, corresponding to the molecular weights of 14.3, 13.1, and 30 kDa, representing BSP1, BSP3, and BSP5, respectively. This study identified BSP proteins as the major seminal plasma protein of Vechur bull seminal plasma.

Keywords: *Vechur, seminal plasma, binder of sperm proteins*

The seminal plasma in ungulates consists of three major classes of proteins namely, sperm adhesins (Topfer-Peterson *et al.,* 1998), cysteine-rich secretory proteins (CRISPs) (Schambony *et al.,* 1998) and fibronectin II (FN-2) type proteins (Ekhlasi-Hundrieser *et al.,* 2002). The FN-2 type

1. *PhD Scholar, Dept. of Animal Reproduction, Gynaecology and Obstetrics, CVAS, Mannuthy*
2. *Associate Professor*
3. *Associate Professor and Head*
4. *Assistant Professor, Dept. of Animal Reproduction, Gynaecology and Obstetrics, CVAS, Pookode*
5. *Professor, Dept. of Veterinary Biochemistry and Associate Dean, CVAS, Mannuthy*
6. *Assistant Professor, Dept. of Animal Genetics and Breeding, CVAS, Mannuthy*

**Corresponding author: sophiaxaviervet@gmail.com, Ph. +919495219359*

proteins, presently known as binder of sperm (BSP) proteins, are characterised by their conserved gelatin-binding FN-2 type module. These proteins constitute the major fraction of seminal plasma proteins in cattle (Manjunath and Sairam, 1987), horses (Menard *et al.,* 2003), goat (Villemure *et al.,* 2003), bison (Boisvert *et al.,* 2004) and buffalo (Harshan *et al.,* 2009; Ramteke *et al.,* 2020).

The major BSP proteins isolated from bovine seminal plasma are BSP1, BSP3 and BSP5. They constitute 40-57 per cent of the total seminal plasma proteins. BSP1 or PDC-109 is the major BSP protein and contributes 25- 47 per cent of the total seminal plasma proteins (Nauc and Manjunath, 2000) and it has been stated to be a multifunctional protein because of its crucial role in various steps of fertilisation such as sperm capacitation (Therein *et al*., 2005), motility (Triphan *et al.,* 2007) and oviductal sperm reservoir formation (Gwathmey *et al*., 2006). Other functional roles suggested for BSP proteins include chaperon like activity (Sankhala *et al.,* 2011), cell volume regulation (Sahin *et al.,* 2009) and antiviral properties (Sperber *et al.,* 2022).

In contrast to their positive role in fertility, BSP proteins may be detrimental to sperm in the context of sperm storage. The cholesterol efflux stimulated by BSP proteins was reported to be time and concentration-dependent (Manjunath and Therien, 2002). Therefore, continuous exposure of sperm to BSP proteins (as in the case of semen collected for *in vitro* storage), causes continuous cholesterol removal from the sperm membrane, which can render sperm sensitive to storage in the liquid or the frozen state (Bergeron *et al.,* 2007).

There is an inadequacy of studies on the quality and composition of Vechur bull seminal plasma protein. This is significant, when considering the fact that the present cryopreservation protocols for Vechur semen follow those adopted for the *Bos taurus* bull semen, without taking into account the possibility of breed differences, especially in seminal plasma proteins. Cryopreservation of semen and its use for artificial insemination (AI) presents a powerful biotechnological tool for the conservation of breeds amenable to AI. The protocols for cryopreservation of semen are influenced by the semen characteristics of the breed. Hence, studies directed at identifying BSP proteins in Vechur semen could help in modifying and improving the existing preservation and AI protocols in Vechur cattle.

Materials and methods

Vechur bulls maintained at the Vechur farm under the Vechur conservation project of the Department of Animal Breeding and Genetics, College of Veterinary and Animal Sciences, Mannuthy were used for semen collection and harvest of seminal plasma proteins. The bulls were screened for breeding soundness and six healthy adult Vechur bulls were selected for the study.

Semen was collected using an artificial vagina as per the standard practice. Immediately after collection, the semen was supplemented with a protease inhibitor cocktail used at the manufacturer's recommended concentrations (P 8340, Sigma Aldrich, USA). Only those ejaculates which had sperm progressive motility of 70 per cent or more were selected. Six ejaculates each were collected from six individual bulls to obtain a total of 36 ejaculates.

Each ejaculate was centrifuged initially at 2000 g for 20 min at room temperature to remove the suspended sperm. The supernatant plasma was collected and further centrifuged at 10000 g for 60 min at 5°C to clarify the seminal plasma of cells, if any. The seminal plasma was supplemented with sodium azide (0.025 per cent) and then stored at –80 °C until analysis. The total protein content of seminal plasma of individual ejaculates from each bull was estimated using the BCA protein assay kit (EMD Millipore Corp.). Equal volume of seminal plasma of individual bulls were pooled and the proteins precipitated with nine volume of cold (-20°C) ethanol, pelleted and suspended in 50mM ammonium bicarbonate. The proteins were then lyophilized in Operon freeze dryer (Model: FDU7003S, Korea) and subsequently stored at -20°C until further analysis.

The BSP proteins from Vechur bull seminal plasma were isolated using Gelatin-Sepharose affinity chromatography as per the method described by Manjunath *et al.* (1987) with slight modifications. Gelatin-Sepharose4B affinity resin (Cytiva) was loaded to the glass column and packing was done till the column reached a height of 5 cm. The column was equilibrated by passing ten bed volumes of equilibration buffer (50mM Tris HCl, 0.15M NaCl, 10mM EDTA, 2mM PMSF and 0.025%NaN_3). A measured quantity of lyophilized seminal plasma protein (20mg) was dissolved in a minimum volume of the binding buffer and it was loaded into the equilibrated column. The column was extensively washed with equilibration buffer to remove un-adsorbed proteins. The adsorbed gelatin binding proteins (BSP) were eluted in 3 mL fractions with elution buffer (50mM Tris-HCl (pH 7.6), 0.15M NaCl, 8M urea, 10mM EDTA, 2mM PMSF 0.025%NaN_3). The protein fractions were analysed for protein content by BCA protein assay. The protein-rich fractions were pooled and loaded into cellulose membrane dialysis tubing (Sigma Aldrich) and extensively dialysed against 20mM Tris-HCl (pH 6.5) with 1 M NaCl for 48h.

Discontinuous SDS- PAGE profiling of the total proteins of Vechur seminal plasma and purified proteins were done according to Laemmli (1970) in a Biorad mini vertical electrophoresis apparatus (mini PROTEAN® tetra cell, USA).

Result and discussion

The ejaculate characteristics observed for the Vechur bulls in the present study are indicated in Table 1. As ejaculates with at least 70 per cent progressive motile sperm alone were considered for the study, the semen quality observed is thus not comprehensive. A mean ejaculate volume of 2.26 ± 0.36 mL was recorded, with the colour of the ejaculate varying from milky to thick milky. The density of the semen ejaculate was found to vary from DDD to DDDD, mass activity ranged from +++ to ++++ and the individual sperm progressive motility (mean ± SE) was observed to be 78.10 ± 1.05 per cent. These findings are similar to the findings of Venkatachalapathy *et al.* (2004), Karthikeyan (2015) and Mahalingeswara (2018).

Vechur bull semen was recorded to have a mean protein concentration of 84.10 ± 2.73 mg/mL with a range of 71.82 ± 2.73 mg/mL to 108.57 ± 3.18 mg/mL among individual bulls as per BCA protein assay in this study. Noolvi (2015) had reported protein concentration of Vechur bull seminal plasma to vary between 77.3 to 106 mg/mL, while Karthikeyan (2015) had noted a mean protein concentration of 84.18 ± 6.92 mg/mL. Mahalingeswara (2018), had recorded higher protein concentration in Vechur bull semen (126.48 ± 7.36 mg/mL). Minor seasonal variation in total seminal plasma proteins have been reported in bovines (Verde *et al.,* 2020), which along with differences in protocols of protein estimation employed, might account for higher seminal plasma protein concentration recorded in Vechur semen by Mahalingeswara (2018).

A mean of 7.58 ± 0.17 mg of protein was recovered by Gelatin-Sepharose affinity chromatography from the 20 mg of total seminal plasma protein loaded. This fraction represented the BSP proteins. In terms of proportion of total protein loaded, the recovered BSP proteins in each run of the column ranged from 35.95 to 40.70 per cent, with an average of 37.89 ± 0.83 per cent. The findings suggested that BSP proteins constituted a major protein fraction of Vechur bull semen. The BSP proteins are

Table1. Semen characteristics of Vechur bull ejaculates (n=36)

S.No	Semen Characteristics	Mean ± SE	Minimum	Maximum
1	Volume (mL)	2.26 ± 0.36	1.1	4.2
2	Colour	-	Milky	Thick milky
3	Density	-	DDD	DDDD
4	Mass activity	-	+++	++++
5	Progressive motility (%)	78.1 ± 1.05	70	85

characterised by gelatin-binding FN2 domains present in them, which confers them with the ability to bind to gelatin (Plante and Manjunath, 2014), hence gelatin-binding proteins represent the BSP protein fraction of the seminal plasma (Manjunath *et al*., 1987). Nauc and Manjunath (2000) used radio immuno assay (RIA) for analysis of BSP content of seminal plasma and concluded that BSP proteins constituted 40-57 per cent of whole seminal plasma protein. They considered RIA to have better sensitivity in determining BSP protein content than affinity chromatography as the latter would involve losses in different stages of processing, which is avoided in direct measurement of protein content with RIA. The efficiency of binding of BSP proteins to the gelatin matrix, the efficiency of elution of bound proteins from the Gelatin-Sepharose column using urea as the eluent and losses during dialysis are factors which could lead to loss of BSP proteins.

Bergeron *et al*. (2005) noted that RSP (gelatin binding protein fraction of ram seminal plasma) represented 65 per cent of total seminal plasma protein. Harshan *et al.* (2009) isolated BSP proteins from crossbred cattle seminal plasma by Gelatin- Sepharose affinity chromatography and recorded that BSP proteins constituted 32.24 per cent of the total seminal plasma proteins, which was comparable to the recovery rate of BSP protein in the current study. Krishnan *et al.* (2018) had recorded that Gelatin binding protein fraction of Malabari buck seminal plasma (GSP) constituted 42.75 ± 6.7 per cent of total seminal plasma proteins in bucks with good semen freezability and 35.0 ± 1.65 per cent of total seminal plasma proteins in bucks with poor semen freezability.

On analysis of SDS-PAGE profile of BSP proteins (gelatin binding fraction), three bands were observed corresponding to the molecular weight of 14.3, 13.1 and 30 kDa representing BSP1, BSP3 and BSP5, respectively (Fig.1). Noolvi (2015), had reported three protein bands in the gelatin binding fraction of Vechur seminal plasma, with molecular weights of 13 kDa, 16.3 kDa and 31.5 kDa corresponding to BSP3, BSP1 and BSP5, respectively. Similar observations were made by Manjunath *et al.* (1987), who reported

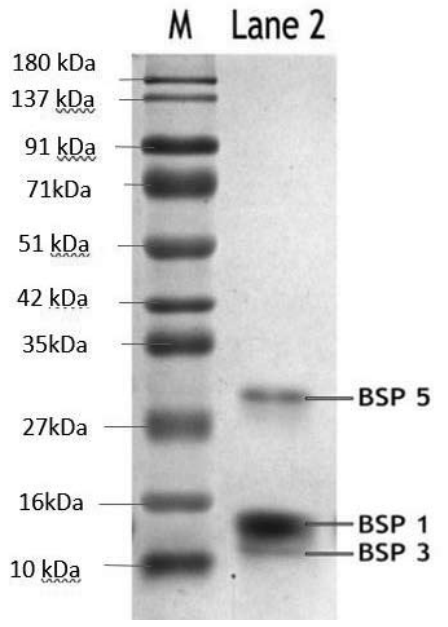

Fig.1 SDS-PAGE profile of purified BSP proteins, Lane 2; Lane M: molecular weight marker (Puregene, 10-180 kDa).

that BSP1 and BSP3 had apparent molecular mass ranging from 15-17 kD and the BSP5 protein had a molecular mass of 28 to 30 kDa. Desnoyers *et al.* (1994) had recorded molecular weight of BSP A1, BSP A2, BSP3 and BSP5 to be 16.5 kDa, 16 kDa, 15 kDa and 28 kDa, respectively.

The BSP1 was found to be doublet, representing BSP A1 and BSP A2, which differed in glycosylation (Nauc and Manjunath, 2000). Manjunath and Sairam (1987) noted that BSP-A1, BSP-A2 and BSP3 migrated as a single distinct band during electrophoresis due to minute variation in molecular weight; and these three proteins alone constituted more than 30 percent of total seminal plasma proteins, resulting in an intense band in SDS-PAGE. Similar findings were also reported by Harshan *et al.* (2009) in buffalo seminal plasma proteins.

The major function associated with BSP protein *in vivo* is its role in sperm capacitation. The proposed mechanism of action of BSP protein on sperm is as follows: upon ejaculation, cauda-epididymal spermatozoa come into contact with seminal

vesicle fluid containing BSP protein. The BSP proteins bind to the choline phospholipids of the sperm membrane and induce cholesterol and phospholipid efflux, destabilising the sperm membrane. The excess BSP proteins are removed by cervical mucus as the sperm travel through the female reproductive tract. Once in the fallopian tube, BSP proteins interact with high-density lipoproteins (HDL) and glycosaminoglycans (GAG) to promote sperm capacitation. HDL acts as an acceptor for the BSP protein, inducing a second phospholipid and cholesterol efflux and destabilising the membrane, leading to capacitation. The interaction of GAG with BSP activates a signalling cascade through tyrosine phosphorylation leading to capacitation (Therien *et al.,* 2005).

Manjunath and Therein (2002) found that BSP-induced cholesterol efflux from spermatozoa was dose and time dependent, with exposure of 15 to 30 min resulting in 7 to 15 per cent cholesterol efflux and exposure of four hours resulting in 25 per cent cholesterol efflux. Although BSP induced significant cholesterol efflux, it was insufficient to complete the process of capacitation. The efflux cholesterol led to sperm membrane destabilisation leading to sperm cryodamage (Harshan, 2007). Bergeron *et al.* (2007) and Singh *et al.* (2007) stated that the protective action of milk casein and egg yolk on sperm cryopreservation were due to their ability to limit BSP mediated sperm cryodamage. Later, Krishnan *et al.* (2018) reported that the deleterious effects of gelatin binding proteins of buck semen (GSP) on sperm cryodamage could be limited by chelating the proteins with choline chloride immediately at ejaculation.

The validation of presence of BSP proteins in Vechur bull seminal plasma, thus opens up the possibilities of modifying Vechur bull semen cryopreservation protocols, so as to limit BSP induced sperm cryodamage and hence improve post-thaw sperm characteristics.

Conclusion

On summarising the results, it could be concluded that BSP constituted a major fraction of seminal plasma proteins of Vechur bulls. As previous studies have indicated deleterious effect of BSP on sperm cryopreservation outcomes, modification of cryopreservation protocols to counter the BSP mediated cholesterol efflux would be of use in improving post-thaw sperm quality.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Bergeron, A., Brindle, Y., Blondin, P. and Manjunath, P. 2007. Milk Caseins decrease the binding of the Major Bovine Seminal Plasma Proteins to sperm and prevent lipid loss from the sperm membrane during sperm storage. *Biol. Reprod.* **77**: 120–126

Bergeron, A., Villemure, M., Lazure, C. and Manjunath, P. 2005. Isolation and characterization of the major proteins of ram seminal plasma. *Mol. Reprod. Dev.***71:** 461–470.

Boisvert, M., Bergeron, A., Lazure, C. and Manjunath, P. 2004. Isolation and characterization of gelatin-binding bison seminal vesicle secretory proteins. *Biol. Reprod*. **70**: 656–661.

Desnoyers, L., Théarien, I. and Manjunath, P. 1994. Characterization of the major proteins of bovine seminal fluid by two-dimensional polyacrylamide gel electrophoresis. *Mol. Reprod Dev.* **37**: 425-435.

Ekhlasi-Hundrieser, M., Sinowatz, F., Greiser De Wilke, I., Waberski, D. and Topfer-Peterson, E. 2002. Expression of spermadhesin genes in porcine male and female reproductive tracts. *Mol. Reprod. Dev.* **61**: 32-41.

Gwathmey, T.M., Ignotz, G.G., Mueller, J.L., Manjunath, P. and 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. *Biol. Reprod*. **75**: 501–507.

Harshan, H. M., Sankar, S., Singh, L. P., Singh, M. K., Sudharani, S., Ansari, M. R., Singh, S. K., Majumdar, A. C. and Joshi, P. 2009. Identification of PDC-109-like protein(s) in buffalo seminal plasma. *Anim. Reprod. Sci.***115**: 306-311.

Harshan, H.M. 2007. Modulation of structural and functional properties of buffalo cauda epididymal spermatozoa by sperm binding seminal plasma protein (PDC-109). *Ph.D. Thesis*, Indian Veterinary Research Institute, Izatnagar, 269p.

Karthikeyan, K. 2015. Isolation and characterization of PDC-109 like protein(s) from Vechur bull seminal plasma. *MSc thesis*. Kerala Veterinary and Animal Sciences University, Pookode, 74p.

Krishnan, K. G., Harshan, H. M., Unnikrishnan, M. P.,Kurien, M. O. and Ramnath, V. 2018. Purification and quantification of fibronectin type II proteins in Malabari buck seminal plasma. *J. Vet. Anim. Sci.***49** (1): 89-92.

Laemmli, V.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*.**227**: 660-685.

Mahalingeswara, H.S. 2018. Effect of gelatin binding proteins (GBP) and non-gelatin binding proteins (NGBP) of seminal plasma on freezability of Vechur bull semen. *M.V.Sc. thesis*. Kerala Veterinary and Animal Sciences University, Pookode. Kerala. 98p.

Manjunath, P. and Sairam, M.R. 1987. Purification and biochemical characterization of three major acidic proteins (BSP-A1, BSP-A2 and BSP-A3) from bovine seminal plasma. *Biochem. J.* **241**:685-692.

Manjunath, P. and Therien, I. 2002. Role of seminal plasma phospholipid-binding proteins in sperm membrane lipid modification that occurs during capacitation. *J. Reprod. Immunol.* **53**: 109–119.

Manjunath, P., Sairam, M.R. and Uma, J. 1987. Purification of four gelatin-binding proteins from bovine seminal plasma by affinity chromatography. *Biosci. Rep.* **7**:231-238.

Ménard, M., Nauc, V., Lazure, C., Vaillancourt, D. and Manjunath, P. 2003. Novel purification method for mammalian seminal plasma phospholipid-binding proteins reveals the presence of a novel member of this family of protein in stallion seminal fluid. *Mol. Reprod. Dev.: Incorporating Gamete Res.* **66**: 349-357.

Nauc, V. and Manjunath, P. 2000. Radio immunoassays for bull seminal plasma proteins (BSP-A1/A2, BSP-A3, and BSP-30-kDa) and their quantification in seminal plasma and sperm. *Biol. Reprod*. **63**: 1058–1066.

Noolvi, A. 2015. Isolation and characterization of major fertility-associated proteins from seminal fluid of Vechur and crossbred bulls. *M.V.Sc Thesis*. Kerala Veterinary and Animal Sciences University, Pookode, 65p

Plante, G. and Manjunath, P. 2014. Binder of sperm (BSP) proteins in the male reproductive tract of farm animals. In *9th Biennial Conference of the Association for Applied Animal Andrology*.

Ramteke, S.S., Rajoriya, J.S., Shende, A.M., Ghosh, S.K., Prasad, J.K., Perumal, P. and Singh, M. 2020. Effect of buffalo PDC-109 on caudal epididymal spermatozoa of bubaline species. *Indian J. Anim. Sci.* **90**: 1584-1588.

Sahin, E., Petrunkina, A.M., Ekhlasi-Hundrieser, M., Hettel, C., Waberski, D., Harrison, R.A. and 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. *Reprod. Fert. Dev.* **21**: 479-488.

Sankhala, R.S., Damai, R.S. and Swamy, M.J.

2011. Correlation of membrane binding and hydrophobicity to the chaperone-like activity of PDC-109, the major protein of bovine seminal plasma. *PLoS One*. **6**: e17330.

Schambony, A., Gentzel, M., Wolfes, H., Raida, M., Neumann, U. and Töpfer-Petersen, E., 1998. Equine CRISP-3: primary structure and expression in the male genital tract. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*. **1387**: 206-216.

Singh, L. P., Harshan, H. M. and Ansari, M. R. 2007. Effect of egg yolk and seminal plasma heparin binding protein interaction on the freezability of buffalo cauda epididymal spermatozoa. *Anim. Reprod. Sci.* **99**: 395-400

Sperber, H.S., Sutter, K., Müller, K., Müller, P. and Schwarzer, R.2022. The Bovine Seminal Plasma Protein PDC-109 Possesses Pan-Antiviral Activity. *Viruses.* **14**: 2031.

Thérien, I. Bergeron, A., Bousquet, D. and Manjunath, P. 2005. Isolation and characterization of glycosaminoglycans from bovine follicular fluid and their effect on sperm capacitation. *Mol. Reprod. Dev.* **71**(1):97-106.

Topfer-Peterson, E., Romero, A., Valera, P.F., Ekhlasi-Hundrieser, M., Dostalova, Z, Sanz, L. and Calvete, J.J. 1998. Spermadhesins: a new protein family. Facts, hypothesis and perspectives. *Andrologia*. **30**: 217-224.

Triphan J, Aumuller G, Brandenburger T and Wilhelm B. 2007. Localization and regulation of plasma membrane Ca (2+)-ATPase in bovine spermatozoa. *Europ. J. Cell Biol*. **86**: 265-273.

Venkatachalapathy, R.T., Kurien, E. and Iype, S. 2004. Studies on semen characteristics of Vechur bulls [abstract]. In: *Compendium, National Symposium on Livestock Biodiversity vis-à-vis Resource Exploitation: an Introspection*; 11th to 12th Febuary, 2004, Karnal. National Bureau of Animal Genetic Resources. p. 205. Abstract No. A.G.R.0.3.6.

Verde, L. I., Nongbua, T., Karkehabadi, S., Johannisson, A. and Morrell, J. M. 2020. Effect of season on bovine seminal plasma proteins in Thailand. *J. Thermal Biol.* **90**: 1-7.

Villemure, M., Lazure, C. and Manjunath, P. 2003. Isolation and characterization of gelatin-binding proteins from goat seminal plasma. *Reprod. Biol. Endocrinol*. **1**: 39.

■