Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 70 (2008) 1352–1355
Theriogenology
www.theriojournal.com

# Major proteins of boar seminal plasma as a tool for biotechnological preservation of spermatozoa

I. Caballero [a,*], J.M. Vazquez [a], E.M. García [a], I. Parrilla [a,c], J. Roca [a], J.J. Calvete [b], L. Sanz [b], E.A. Martínez [a]

[a] *Department of Animal Medicine and Surgery, University of Murcia, Murcia Spain*
[b] *Institute of Biomedicine, C.S.I.C., Valencia, Spain*
[c] *Institute for Animal Breeding, Mariensee (FAL), Germany*

**Abstract**

Boar seminal plasma is a complex mixture of secretions from the testes, epididymides, and the male accessory reproductive organs which bathe the spermatozoa at ejaculation. The seminal plasma contains factors, mostly proteins, which influence the spermatozoa, the female genital tract, and the ovum. In boars, most of the proteins belong to the spermadhesin family and bind to the sperm surface. Spermadhesins are multifunctional proteins with a wide range of ligand-binding abilities to heparin, phospholipids, protease inhibitors and carbohydrates; the family can be roughly divided into heparin-binding (AQN-1, AQN-3, AWN) and non-heparin-binding spermadhesins (PSP-I/PSP-II heterodimer). These proteins have various effects promoting or inhibiting sperm functions including motility, oviduct binding, zona binding/penetration, and ultimately fertilization. The complexity of the environmental signals that influence these actions have implications for the uses of these proteins *in vivo* and *in vitro*, and may lead to uses in improving sperm storage.


*Keywords:* Seminal plasma protein; Spermadhesin; Sperm capacitation; Oviductal sperm reservoir; Boar semen

## 1. Introduction

Boar seminal plasma (SP) is a complex mixture of secretions originating from the testes, epididymides, and the male accessory reproductive organs in which the spermatozoa are bathed at ejaculation. It is well established that SP contains factors that influence both the spermatozoa and the female genital tract during sperm transport [1–4]; most of these factors are SP proteins. In boars, the bulk of SP proteins (>90%) belong to the spermadhesin family, a group of 12–16 kDa glycoproteins that bind to the sperm surface. These proteins, containing a single CUB domain architecture [5,6], comprise five members: AQN-1, AQN-3, AWN, PSP-I and PSP-II. The latter two proteins form a glycosylated PSP-I/PSP-II heterodimer and represent over 50% of the total protein content in boar SP. Spermadhesins are multifunctional proteins exhibiting a wide range of ligand-binding abilities to heparin, phospholipids, protease inhibitors and carbohydrates that change with glycosylation and aggregation states. Depending on their binding capability, spermadhesins can be classified into heparin-binding (AQN-1, AQN-3, AWN) and non-heparin-binding spermadhesins (PSP-I/PSP-II heterodimer) [6–12].

* Corresponding author at: Departamento de Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad de Murcia, 30071 Murcia, Spain. Tel.: +34 968 364812; fax: +34 968 367069.
*E-mail address:* nachof@um.es (I. Caballero).

0093-691X/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2008.07.013

## 2. The role of spermadhesins during fertilization

The variety of ligand-binding properties suggests that spermadhesins play a role in several events during the sperm lifespan including sperm capacitation, formation of the oviductal sperm reservoir, gamete interaction, and uterine immunomodulation. It has been suggested that after ejaculation, monomeric forms of AWN-1 and AQN-3 that exhibit specific binding to phospholipids, form a first layer of coating material by interacting with the lipid bilayer of the sperm membrane [13]. Thereafter, the spermatozoa would be coated by a layer of aggregated spermadhesins which serve as acrosome-stabilizing factors [6,13]. Once the ejaculated is deposited in the female genital tract, there is a massive invasion of leukocytes into the uterine lumen that can be modulated by the PSP-I/PSP-II heterodimer and its subunits [14–17]. The surviving sperm population that reaches the oviduct colonizes the isthmic reservoir by means of carbohydrate-based interactions between sperm surface proteins and the oviductal epithelium [18]. Recently, heparin-binding spermadhesins (especially AQN-1) and their aggregated forms, have been seen to interact with the oviductal epithelium, suggesting a contribution of these spermadhesins in the formation of the oviductal sperm reservoir [19,20]. The sperm reservoir preserves the fertilizing capacity of spermatozoa and modulates sperm capacitation [17].

The beginning of *in vitro* sperm capacitation leads to both the removal of AQN-type proteins from the sperm surface and a significant decrease in the ability of spermatozoa to bind to oviductal explants [19]. Moreover, most of the spermadhesins are released from the sperm head during *in vitro* capacitation. The cholesterol-binding ability of aggregated spermadhesins points to these proteins becoming acceptors of cholesterol molecules, suggesting a role in ''cholesterol efflux'', a key event during capacitation [12,21].

Regarding gamete binding, *in vitro* assays have demonstrated the affinity of AWN, AQN-1 and AQN-3 for zona pellucida [12]. Both AWN and AQN-3 are tightly interconnected with the phospholipids of the sperm membrane bilayer. Thus, they are still in position to act as a receptor for the zona pellucida. Moreover, that sperm-bound AWN reaches pig oocytes after *in vivo* insemination, suggested a role of the spermadhesins in sperm–oocyte interaction [22,23].

## 3. Application of spermadhesins to reproductive biotechnologies

During recent years, there has been a considerable interest in the development of emerging reproductive biotechnologies, such as cryopreservation and sex-sorting of spermatozoa, that would enhance the profitability of the swine industry [24,25]. However, that these procedures involve sperm injury, caused by the removal or extreme dilution of SP, hampers their practical application. Although, a certain proportion of SP added to sperm suspensions has been postulated as a counter-measure to alleviate the injury to spermatozoa produced by such reproductive biotechnologies [26,27], differences in SP effect (beneficial or detrimental) have been reported. These differences may be related to a variation in the presence, absence or concentration of SP factors, mainly proteins [28–31].

The high variability of SP composition [31] prompted our research group to study the effect of specific protein fractions on sperm technologies. Supplementation of boar spermatozoa subjected to extreme dilution (similar to that occurring during flow-cytometric cell sorting for sex preselection) with heparin-binding (HBP) and non-heparin-binding (PSP-I/PSP-II heterodimer) spermadhesins exerted opposite effects on sperm viability, motility, and mitochondrial activity. Although HBP had a markedly time- and concentration-dependant detrimental effect, PSP-I/PSP-II preserved the functionality of highly diluted boar spermatozoa [32]. The beneficial effect of PSP-I/PSP-II was concentration-dependent, with an optimal concentration of 1.5 mg/mL (equivalent to 10% raw SP). The use of an isolated protein would avoid the inherent variability of protein content in whole SP [32]. Dissection of the PSP-I/PSP-II heterodimer in its isolated PSP-I and PSP-II subunits, and their derived peptidic and glycan fractions, revealed that the protective effect of the heterodimer is largely preserved in the isolated PSP-II subunit. This protective effect does not appear to require its glycan moiety pointing suggestion that a peptide moiety could be used as the sperm function-preserving additive for highly diluted boar spermatozoa [33].

Based on immunolocalization studies, PSP-I/PSP-II heterodimer is located mainly on the acrosomal region of the sperm head. Long-term incubation (10 h) of highly diluted boar spermatozoa caused a redistribution of the heterodimer from the acrosome to the post-acrosomal domain, and ultimately removal from the sperm surface. However, when highly diluted boar spermatozoa were incubated in the presence of 1.5 mg/