Theriogenology 79 (2013) 1071–1082

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Boar seminal plasma exosomes: Effect on sperm function and protein identification by sequencing

Lidia L. Piehl [a,*], M. Laura Fischman [b], Ulf Hellman [c], Humberto Cisale [b], Patricia V. Miranda [d,1]

[a] Cátedra de Física and Instituto de Bioquímica y Medicina Molecular, IBIMOL (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina
[b] Laboratorio de Calidad Seminal y Criopreservación de Gametas, Cátedra de Física Biológica, INITRA, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Buenos Aires, Argentina
[c] Ludwig Institute for Cancer Research, Uppsala, Sweden
[d] Instituto de Biología y Medicina Experimental-CONICET, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 22 June 2012
Received in revised form 29 January 2013
Accepted 30 January 2013

Keywords:
Exosome
Prostasome
Sperm capacitation
Boar
Seminal plasma

ABSTRACT

Mammalian seminal plasma contains membranous vesicles (exosomes), with a high content of cholesterol and sphingomyelin and a complex protein composition. Their physiological role is uncertain because sperm stabilization and activation effects have been reported. To analyze a putative modulatory role for semen exosomes on sperm activity in the boar, the effects of these vesicles on several sperm functional parameters were examined. Additionally, boar exosome proteins were sequenced and their incorporation into sperm was explored. Boar sperm were incubated under conditions that induce capacitation, manifested as increased tyrosine phosphorylation, cholesterol loss and greater fluidity in apical membranes, and the ability to undergo the lysophosphatidylcholine-induced acrosome reaction. After establishing this cluster of capacitation-dependent functional parameters, the effect produced by exosomes when present during or after sperm capacitation was analyzed. Exosomes inhibited the capacitation-dependent cholesterol efflux and fluidity increase in apical membranes, and the disappearance of a 14-kD phosphorylated polypeptide. In contrast, the acrosome reaction (spontaneous and lysophosphatidylcholine-induced) was not affected, and sperm binding to the oocyte zona pellucida was reduced only when vesicles were present during gamete coincubation. Liposomes with a lipid composition similar to that present in exosomes mimicked these effects, except the one on zona pellucida binding. Interaction between exosomes and sperm was confirmed by transfer of aminopeptidase activity. In addition, the major exosome protein, identified as actin, appeared to associate with sperm after coincubation. Exosome composition had a predominance for structural proteins (actin, plastin, ezrin, and condensin), enzymes, and several porcine seminal plasma-specific polypeptides (e.g., spermadhesins). Transfer of proteins from exosome to sperm and their ability to block cholesterol efflux supports a direct interaction between these vesicles and sperm, whereas inhibition of some capacitation-dependent features suggests a stabilizing function for exosomes in boar semen.

1. Introduction

Mammalian sperm leaving the testis are morphologically differentiated, but immotile and unable to fertilize the oocyte. They must undergo several morphological and functional changes to become fully fertile. The first stage,

* Corresponding author. Tel./fax: +54 11 4964 8201.
E-mail address: lpiehl@ffyb.uba.ar (L.L. Piehl).
[1] Present address: Instituto de Agrobiotecnología Rosario (INDEAR), Santa Fe, Argentina.

0093-691X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2013.01.028

known as maturation, takes place during sperm transit through the epididymis, where they experience an extensive plasma membrane remodeling that involves acquisition and redistribution and release of various components, including lipids and proteins. As a consequence of this process, sperm acquire progressive motility and a potential ability to recognize and fertilize an oocyte [1]. However, additional functional maturation steps must be completed for sperm to be able to fully express these capabilities. Sperm are stored in the terminal portion of the epididymis waiting for the appropriate signal that will cause their release at ejaculation. At that time, cells come into contact with accessory sex gland secretions and are deposited in the female reproductive tract, where they undergo several structural and functional changes that render them ready to find, recognize, penetrate, and fertilize an oocyte. This complex process is known as sperm capacitation and can be reproduced *in vitro* by sperm incubation under adequate conditions [1–3]. Capacitation is a complex cascade of molecular events that includes cholesterol efflux with the consequent modification of sperm membrane composition and fluidity [4,5], phospholipid scrambling [6], changes in intracellular ion concentrations [7], and increased tyrosine phosphorylation in several proteins [8]. The functional consequences of all these processes are reflected in the ability of sperm to undergo the acrosome reaction (AR), and acquisition of a distinctive pattern of motility known as hyperactivation [1].

Ejaculation and capacitation are intimately related, not only chronologically, but also functionally. It is assumed that accessory sex gland secretions stabilize sperm for their transit along the female tract. The ability of seminal plasma to prevent and revert capacitation was reported together with the description of this event [9]. This effect was later connected to inhibition of the induced AR [10,11] and tyrosine phosphorylation of sperm proteins [12]. Cholesterol was indicated as the probable cause, because it could reproduce the effects of seminal plasma [10,13].

Mammalian seminal plasma contains membranous vesicles (exosomes) characterized by a high cholesterol and sphingomyelin content, and a complex protein composition [14–18]. These vesicles are produced by the epididymis and the prostate [19]. Prostasomes, the membrane vesicles secreted by the human prostate, have been more extensively studied [20]. In addition, similar vesicles have also been isolated from the seminal plasma of rat, rabbit, ram, bull, stallion, and boar [16,18,21–24]. Because prostasomes have immunosuppressive, antioxidant, and antibacterial properties, it has been suggested that they are involved in several biological processes which can indirectly influence sperm function [20]. Regarding a direct action, it is known that human prostasomes can interact with sperm; however, the purpose and relevance of this interaction is still controversial, because activating and stabilizing effects have been postulated. Vesicles isolated from rabbit seminal plasma inhibit fertility [22]. Conversely, prostasomes were reported to promote forward motility of human sperm [25,26]. With regard to the AR, several groups studied the effect of prostasomes with diverse results [24,27–30]. Recently, it was reported that prostasomes can affect the tyrosine phosphorylation of sperm proteins [29,31]. However, a wide study on the possible role of exosomes on different aspects of sperm function is still lacking. Only a few studies on the effects of exosomes on sperm capacitation are available, but none have been conducted in the boar.

In the present study, the effect of exosomes isolated from boar seminal plasma on cholesterol efflux, membrane fluidity, protein tyrosine phosphorylation, AR, and binding to oocytes were analyzed to determine a possible modulatory role for these vesicles on sperm function. Additionally, boar exosome proteins were identified by sequencing, and their incorporation into sperm was explored.

2. Materials and methods

2.1. Chemicals

All reagents used were of high purity or analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA), Fisher Scientific (Loughborough, Leicester, UK), Merck (Darmstadt, Hesse, Germany), or J.T.Baker (Phillipsburg, NJ, USA).

2.2. Samples

Semen samples were obtained by the standard gloved-hand technique from five adult hybrid boars (cross of three pure breeds: Large White, Pietrain, and Hampshire) housed at an artificial insemination center in the School of Veterinary Sciences of the University of Buenos Aires. Handling of animals was in accordance with the principles expressed in the "Legislation for the protection of animals used for scientific purposes" (European Commission).

Pre- and post- sperm-rich fractions were discarded, and the sperm-rich fraction was used for analysis. The following parameters were measured to determine semen quality: ejaculate volume, sperm viability, motility, concentration, morphology, and response in the hyposmotic swelling test. Only samples which met the following quality requirements were used: volume greater than 50 mL, progressive motility greater than 70%, abnormal sperm less than 20%, and concentration of at least 3×10^8 sperm per mL. Ejaculates were processed individually.

2.3. Sperm incubation

The sperm-rich fraction was diluted (1.5×10^7 cells per mL) in Tyrode's medium (100 mM NaCl, 3.1 mM KCl, 0.4 mM $MgSO_4$, 0.3 mM NaH_2PO_4, 5 mM glucose, 20 mM HEPES, 1 mM sodium pyruvate, 21.7 mM sodium lactate, 15 mM $NaHCO_3$ and 2 mM $CaCl_2$, pH 7.4) supplemented with 3 mg/mL BSA. Sperm were then incubated at 39 °C in a 5% CO_2 humidified atmosphere for up to 3 hours. To evaluate the effect of exosomes on different sperm functions, two experimental approaches were tested: vesicles were added either at the beginning or during the last 30 minutes of incubation. In a parallel set of experiments, sperm were incubated in a similar manner with liposomes with a lipid composition similar to exosomes. Sperm motility was estimated at the end of the incubation using a phase-contrast light microscope (magnification × 400) with a thermal stage (37 °C).