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Evidence of the Expression of Basigin in Mature Porcine Sperm

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Abstract

The presence of the protein Basigin on the surface of ejaculated mature porcine sperm was identified for the first time. Sperm proteins were separated by electrophoresis and identified by western blot with commercial EMMPRIN Antibody. Boar sperm specific antibodies were developed as positive control. Basigin has been found in many cells of diverse species and it would be related to the processes of sperm-oocyte binding.

Keywords: *BASIGIN – SPERM - BOAR.*

Introduction

Spermatogenesis consists on the daily production of sperm by the testis. It involves processes of migration, cell differentiation and presents a strict hormonal regulation (Shima et al., 2004).

The metalloproteases of the extracellular matrix (MMP) play a central role in the repair of tissues subjected to injury and in the restoration of homeostasis between the epithelium and its stroma. MMP production and activation is regulated by cytokines, growth factors and hormones. Among the MMP-inducing factors, a glycosylated transmembrane protein called CD147 / EMMPRIN has been identified (Ellis et al., 1989; Kataoka et al., 1993; Taylor et al., 2002). EMMPRIN has a wide range of tissue distribution and its expression has been detected in activated T lymphocytes (Kasinrerk et al., 1992), in differentiated macrophages (Major et al., 2002), in the pigmented retinal epithelium (Marmorstein et al., 1998) and in the endometrium, among others (Noguchi et al., 2003).

The cDNA of human EMMPRIN codes for a protein that belongs to the immunoglobulins superfamily (Biswas, et al. 1995; Yoshida et al., 2000), and its sequence has been identified as identical to that of the human Basigin (Miyauchi et al., 1991). EMMPRIN homologous proteins have been described in other species such as Basigin (Bsg) or gp 42 in mouse (Miyauchi et al., 1991), OX47 in rat (Fossum et al., 1991; Nehme et al., 1995), and 5A11, HT7, or neurothelin in birds (Seulberger et al., 1992; Fadool & Linser, 1993). Basigin is composed by two immunoglobulin-like domains in the extracellular region, a transmembrane domain and a short intracytoplasmic portion corresponding to 39 amino acids (Miyauchi et al., 1991; Biswas et al., 1995; Muramatsu & Miyauchi, 2003). The extracellular region contains 3 asparagine corresponding to potential glycosylation sites (Muramatsu & Miyauchi, 2003). The level of glycosylation depends on the molecular origin and its biological activity. The molecular weight is variable and has a range of 44 to 66 kDa (Biswas et al., 1995). The intracellular portion is conserved among species, indicating thatit could be involved in the translation of intracellular signals (Miyauchi et al., 1991).

Basigin participates in spermatogenesis (Matzuk & Roy, 2006), in embryonic implantation (Saxena et al., 2002) and in the sperm-ovocite interaction (Kuno et al., 1998). It is also expressed in a variety of cancers, during development processes, wound healing, nutrient

transport, inflammatory processes, atherosclerosis, arthritis and microbial pathologies(Agrawal & Yong, 2011). As a member of the immunoglobulin superfamily, it plays a fundamental role in the intercellular recognition and activation of T cells (Koch et al., 1999). Moreover, it has been described that it interacts with the neuroglia forming part of the blood-brain barrier (Koch et al., 1999; Mamoriet al., 2007). In murine testis, CD147 is expressed in Sertolicells, Leydig cells and in all germ cell stages. The *knockout* for Basigin male mouse is sterile, the germ cells are arrested in the spermatid stage (Chen et al., 2011; Bi et al., 2013). Besides, these animals present a decrease of N-cadherins in the basal compartment of the seminiferous tubules, causing disruption of the integrity of the hematotesticular barrier (Bi et al., 2013).

Until now, the presence of Basigin in boar spermatozoa has not been described. The goal of this work was to identify the presence of the glycoprotein Basigin in ejaculated porcine sperm.

Materials and Methods

The ejaculates were obtained from adult hybrid boars that were housed in the Facultad de Ciencias Veterinarias of the Universidad de Buenos Aires. The animals were fed with corn and soy, and received water *ad libitum*. The ejaculates were collected by the gloved hand technique (Hancock & Howell, 1959). The semen was tempered in a thermostatic bath at 37 °C for 30 minutes. It was diluted 1:2 incommercial diluent Androstar™, and the samples were stabilized for 10 minutes at 37 °C. After that time, they were tempered at 20ºC for 20 minutes more. Then, the samples were stabilized at 17ºC for an hour and centrifuged at 1000 rpm for 15 minutes, at the same temperature. The supernatant was discarded and the sperm pellet was resuspended in PBS to a final volume of 10 ml.

Protein expression analysis was performed by Western Blot technique using as a primary antibody the commercial reagent EMMPRIN Antibody (N-19) sc-9752 (Santa Cruz Biotechnology, Inc™) corresponding to an IgG goat polyclonal that recognizes specifically an epitope located near N-terminus of EMMPRIN of human origin. As a secondary antibody, rabbit anti-goat IgG antibody labeled with horseradish peroxidase at a 1:500 dilution(HRP) (KPL™ Laboratories) was used.

In order to control the antigen, a mouse immunization schedulewas prepared to obtain a porcine sperm control serum. Two pelletsof 300 µg of spermatozoa diluted in PBS and emulsified with incomplete Freund's adjuvant were inoculated subcutaneously to the mice, with 15 days interval. The mice serum was obtained 25 days post-last inoculation.

25 µg of protein of sperm samples added with 0.2 mmol phenyl-methyl-sulfonyl-fluorinated-orthovanadate, 1.0 µg pepstatin, 1 µg leupeptin (Sigma™) as antienzymatic, and Triton 0.1% v / v in PBS were subjected to SDS-PAGE.

The samples were heated at 100 °C for 5 minutes in buffer and plated 20 µl in a 12% polyacrylamide gel. Electrophoresis was performed at 100 volts for 2 hours.

As a positive control of the primary antibody, 20 µg of a mouse myeloma cell line Sp2 / 0 was used, the negative control was without the primary antibody, and antigen control was using boar spermatozoa.Once the electrophoresis was finished, the nitrocellulose membrane was allowed to equilibrate for 30 minutes at room temperature in a transfer buffer solution.

The transfer was carried out in a refrigerated room for 16 hours at 30 volts, with buffer agitation in BIORAD Transblot transfer tub. Subsequently, the membrane was blocked with PBS-10% milk for 1 hour. Then, three washes of 10 minutes were made in agitation with PBS.

The primary polyclonal anti-basigin antibody from porcine anti-spermatozoon mice diluted 1:200 in PBS-5% milk was placed for one hour. Three consecutive washes were made with PBS Tween of 3 minutes each. The HRP anti-goat antibody conjugate (1:2000) was placed in the anti-basigin treated membrane or the anti-mouse conjugate produced in goat labeled with HRP (KPL Laboratories™), for 1 hour at room temperature under constant stirring in dilution 1:500 in PBS-5% milk.

After the incubation, two washes were made with PBS Tween and the reaction was revealed with Di-amino-benzidine (DAB) (Sigma™), and stopped with distilled water.

Results

It was possible to identify that the human-specific anti-Basigin antibody recognizes at least two molecular

weight bands between 62 and 47, between 47 and 32 kDa and others of lower weight with weaker signals in porcine sperm from fresh ejaculate. The results are shown in Figure 1.

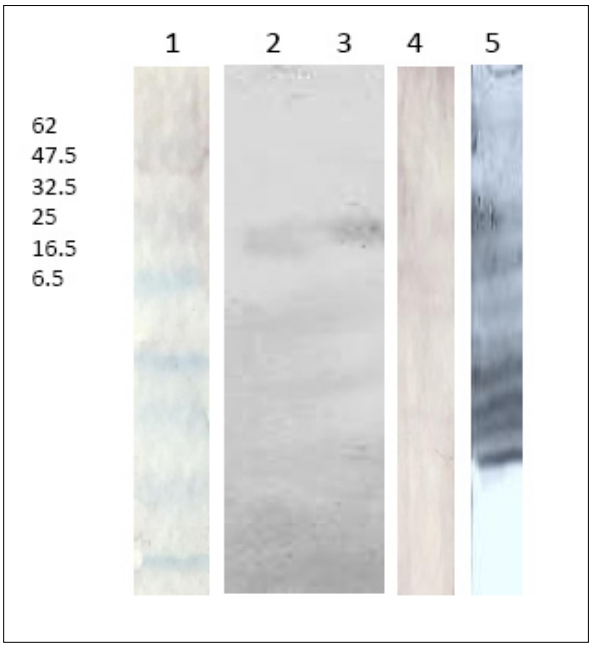

Figure 1. *Molecular weight Broad Range 2: porcine sperm with EMMPRIN Antibody (N-19) sc-9752, 3: positive control of reactivity of EMMPRIN Antibody (N-19) sc-9752 with mieloma Sp2/0, 4: negative control porcine sperm with anti-goat HRP, 5: porcine sperm control sperm reacted with anti-porcine sperm mouse polyclonal sera.*

Discussion and Conclusions

The potential role of Basigin (homologue of EMMPRIN) in the murine species has been widely studied (Iacono et al. 2007; Gabison et al. 2005) In a mutant Basigin deficient mice model, embryos are lost close to implantation and those that survive in adulthood are sterile (Bi et al., 2013). Male infertility is related to defects in sperm differentiation, because of the high expression of Basigin detected in testicular germ cells, from animals without mutations, and the different levels of glycosylation, which are related to sperm during spermiogenesis (Chen et al., 2011; Bi et al., 2013). The glycosylation of EMMPRIN seems to represent a post-transcriptional regulatory mechanism in the normal physiological process (Biswas et al., 1995).

In this work, it was possible to detecta protein that,-to our knowledge,- had not been identified in boar sperm, thus contributing to the knowledge of essential proteins in fertility.The polyclonal anti-Basigin antibody allowed to identifya series of signals in the extract of boar sperm from fresh ejaculates. This antibody has been used as a positive control in tumor cells experiments (squamous cell carcinoma, mammary adenocarcinoma, lung carcinoma, etc.) and not in sperm cells. This indicates that the studies should be deepened to identify the presence of a homologous protein in the boar sperm and to know the importance of its expression in the spermatogenesis process. For this, trials should be designed with the use of mass spectrometry, HPLC and the possible sequencing of chains of this protein.

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