Zygote **23** (April), pp. 229–236. © Cambridge University Press 2013
doi:10.1017/S0967199413000464 First Published Online 23 October 2013

S100A7 in the Fallopian tube: a comparative study

Juan M Teijeiro[2] *and Patricia E. Marini*[1,3]
Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario; and Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET, Rosario, Argentina

Date submitted: 30.04.2013. Date revised: 01.08.2013. Date accepted: 09.09.2013

Summary

The oviduct is a dynamic organ in which final gamete maturation, fertilization and early embryo development take place. It is considered to be a sterile site; however the mechanism for sterility maintenance is still unknown. S100A7 is an anti-microbial peptide that has been reported in human reproductive tissues such as prostate, testicle, ovary, normal cervical epithelium and sperm. The current work reports the presence of S100A7 in the Fallopian tube and its localization at the apical surface of epithelial cells. For comparison, porcine S100A7 was used for antibody development and search for peptide in reproductive tissues. Although present in boar seminal vesicles and seminal plasma, S100A7 was not detected on female porcine organs. Also, in contrast with the human protein, porcine S100A7 did not show anti-microbial activity under the conditions tested. Phylogenetic analyses showed high divergence of porcine S100A7 from human, primate, bovine, ovine and equine sequences, being the murine sequence at a most distant branch. The differences in sequence homology, *Escherichia coli*-cidal activity, detectable presence and localization of S100A7 from human and pig, suggest that there are possible different functions in each organism.

Keywords: Fallopian tube, Oviduct sperm, S100A7, Seminal plasma

Introduction

In order for mammalian fertilization to proceed sperm must enter the female reproductive tract, where the selection processes submit them to strict controls (Hunter, 2005; Suárez, 2007). The subpopulation of sperm that reach the oviduct can bind to the epithelial cells of the isthmus, as occurs in several mammals such as human, sheep, cow, horse, pig and mouse (Hunter, 1981; Flechon & Hunter, 1981; Hunter & Nichol, 1983; Hunter & Wilmut, 1984; Demott & Suárez, 1992; Thomas *et al.*, 1994; Pacey *et al.*, 1995; Suárez, 1998); such bound sperm show decreased movement and prolonged survival (Fazeli *et al.*, 2003; Suárez, 2008). It has been demonstrated that oviductal proteins can modulate those sperm functions (Avilés *et al.*, 2010; Holt & Fazeli, 2010; Hung & Suárez, 2010; Killian, 2011). Oviductal annexin A2 has been involved in the attachment of sperm to oviductal epithelium in pig (Teijeiro *et al.*, 2009) and cow (Ignotz *et al.*, 2007) and oviductal porcine deleted in malignant brain tumour 1 (DMBT1) has been related to sperm selection (Teijeiro & Marini, 2012a; Teijeiro *et al.*, 2008, 2011, 2012).

However, the female genital tract is a complex biosystem that is constantly exposed to a multitude of bacterial, fungal and viral agents (Mildner *et al.*, 2010). Importantly, anti-microbial peptides (AMPs) are small proteins produced by epithelial surfaces and inflammatory cells that have broad-spectrum anti-microbial and immunomodulatory activities and are present in a number of sites throughout the female reproductive tract (Frew & Stock, 2011). S100A7 (Psoriasin), a protein that belongs to a multigenic family of calcium-modulated proteins of the EF-hand type, has been identified as an AMP with strong

[1]All correspondence to Patricia E. Marini. Área Biología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 590, Rosario S2002LRK, Argentina. Tel: +54 341 4350661. Fax: +54 341 4804601. e-mail: marini@ibr.gov.ar or pmarini@fbioyf.unr.edu.ar
[2]Área Biología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 590, Rosario S2002LRK, Argentina.
[3]Instituto de Biología Molecular y Celular de Rosario, IBR-CONICET, Rosario, Argentina.

https://doi.org/10.1017/S0967199413000464 Published online by Cambridge University Press

activity against *Escherichia coli* (Gläser *et al*., 2005). Moreover, S100A7 has been proposed as the major *Escherichia coli*-cidal factor in the female genital tract, and is expressed in vulva, vagina and ectocervical epithelia (Mildner *et al*., 2010). In addition, expression of S100A7 has been found in reproductive tissues such as prostate, testicle, ovary and normal cervical epithelium (Shadeo *et al*., 2007). The present authors have identified S100A7 in human and boar sperm and have demonstrated the interaction of porcine sperm S100A7 with porcine oviductal DMBT1 (Teijeiro & Marini, 2012b).

As AMPs are likely to have important functions in the female reproductive tract, and the expression of S100A7, an AMP, was not evaluated in Fallopian tubes, which are considered to be 'sterile sites' (Quayle, 2002), it was decided to search for the presence of this protein in the human oviduct.

Comparatively, the presence of S100A7 in porcine reproductive tissues was also evaluated. Because the *Escherichia coli*-cidal activity of S100A7 has been proposed in mammals other than human, such as cows (Regenhard *et al.*, 2009), anti-microbial activity and further sequence homology analysis of porcine S100A7 were performed.

Materials and methods

Chemicals

Chemicals were obtained from Sigma-Aldrich (Buenos Aires, Argentina), unless otherwise stated. The following primary antibodies were used: polyclonal anti-human S100A7 purified by affinity to His-6-S100A7 immobilized on nitrocellulose membranes (Teijeiro & Marini, 2012b) (dilution 1:1000 for western blot and 1:100 for immunofluorescence) and polyclonal anti-porcine S100A7 (dilution 1:1000). The following secondary antibodies were used: horseradish peroxidase-conjugated anti-rabbit IgG (dilution 1:10,000) (GE Healthcare, Buenos Aires, Argentina); Cy3-conjugated anti-rabbit immunoglobulin (dilution 1:1000) (Chemicon International Inc., Temecula, USA).

Sample collection

Boar seminal plasma was collected from adult fertile boars from different breeds by the glove-handed method. The sperm-rich fraction was separated from seminal plasma by centrifugation. Porcine tongues, testicles, epididymis, seminal vesicles, ovaries, oviducts and uteri were obtained from a local abattoir and transported immediately to the laboratory in ice-cold phosphate-buffered saline (PBS). Each tissue was dissected and cut into pieces 1 × 1 cm. Then, each fragment was resuspended in buffer that contained 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 1% NP40 and dithiothreitol (DTT) 100 mM, and disaggregated by forceps and scissors. The tissue was homogenized in a Potter homogenizer and then sonicated and centrifuged 15 min at 4 °C at 16,000 *g*. The supernatant was collected and stored at –80 °C until use. Human seminal plasma and Fallopian tubes were gifted by Dr Sergio Ghersevich from the Clinical Biochemistry Area, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina. For human samples, study protocols were approved by the Institutional Bioethical Board of Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina and written consent was obtained from all donors. Human oviductal tissue was obtained from premenopausal women ($n = 6$, average age: 42.9 years) with no clinical history of infection or neoplasic diseases, scheduled for hysterectomies as a result of uterine fibromyomas or hypermenorrhea (Hospital Provincial del Centenario, Rosario, Argentina). Samples for western blot were processed in accordance with Zumoffen *et al*. (2013), briefly, fragments of the oviducts were opened longitudinally and the epithelial layer of cells was carefully scraped out with a scalpel and homogenized in homogenization buffer that consisted of 50 mM Tris–HCl (pH 7.4), 0.1 mM EDTA, 0.1 mM EGTA, 0.1 M phenylmethanesulfonyl fluoride (PMSF), 2 μg/ml Aprotinin and 0.1% v/v 2-mercaptoethanol, in an ice bath. The oviductal cells homogenates were then centrifuged at 15,000 *g* for 30 min at 4 °C, and the supernatants stored at −20 °C until further use. Fallopian tube samples for immunofluorescence were provided as paraffin blocks.

Immunofluorescence

Human Fallopian tube tissues were sectioned, deparaffinized in xylene, and then rehydrated through graded dilutions of ethanol (100, 95, 70 and 35%), followed by two washes in Tris-buffered saline (TBS) (25 mM Tris–HCl pH 7.5, 150 mM NaCl). Slides were gently rinsed twice with TBS, blocked with 2% BSA, 0.2% Triton X-100 in TBS for 1 h and treated with the primary antibodies overnight at 4 °C. After rinsing twice with TBS, the slides were treated with Cy3-conjugated anti-rabbit immunoglobulin (1:2,000) for 1h. After rinsing twice with TBS, slides were covered with 4′,6-diamidino-2-phenylindole (DAPI) for 30 min, then were washed with TBS, covered with 0.22 M 1,4-diazabicyclo[2,2,2]octane dissolved in glycerol:TBS (9:1) and cover slips were applied. The preparations were examined under a confocal microscope (Nikon Model Eclipse TE-2000-E2, Melville, USA).

https://doi.org/10.1017/S0967199413000464 Published online by Cambridge University Press

Subcloning and expression of porcine S100A7, and development of anti-porcine S100A7 antibodies (anti-His6-pS100A7)

The coding sequence for porcine S100A7 (pS100A7) was cloned into pBluescript II by Gorodkin *et al*. (2007) and was a gift from Dr Claus Jørgensen (Faculty of Life Sciences, University of Copenhagen, Denmark). This sequence was subcloned into pET-tev vector as histidine fusion and the recombinant protein (His6-pS100A7) was expressed in *E. coli* BL21. Protein expression was induced with isopropyl-1-thio-β-D-galactopyranoside (IPTG) for 20 h and purification was performed with Ni-NTA agarose (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. His6-pS100A7 was eluted five times sequentially with 250 mM imidazole; 0.5 ml fractions were collected and subsequently tested for purity by silver-stained SDS-PAGE (Rabilloud *et al*., 1994). Fractions that contained His6-pS100A7 were pooled and concentrated by ultrafiltration using Ultracel-3 K (Amicon® Ultra, Millipore. Massachusetts, USA) and re-buffered into PBS (130 mM NaCl, 2.68 mM KCl, 6.46 mM Na_2HPO_4, 1.47 mM KH_2PO_4), pH 7.4.

Purified recombinant protein was used for anti-serum development in rabbit as described by Pérez *et al.* (2006). Polyclonal anti-human S100A7 antibodies (anti-hS100A7) were developed in rabbit using recombinant GST-hS100A7 expressed and purified as described by Teijeiro & Marini (2012a).

Western blots

Protein extracts obtained as mentioned above were supplemented with loading buffer (25 mM Tris–HCl, pH 6.8, 30% glycerol, 4% SDS) boiled for 5 min, used for 15% SDS-PAGE and then proteins were transferred to nitrocellulose membranes (GE Healthcare, Buenos Aires, Argentina). Non-specific binding sites were blocked by incubation with 5% dried non-fat milk in TBS (25 mM Tris–HCl, pH 7.4, 150 mM NaCl). Membranes were treated 1 h with the corresponding antibodies. After washing (three times for 10 min each), the blots were incubated with peroxidase-conjugated anti-rabbit IgG for 1 h and washed again. Labelled proteins were revealed using enhanced chemiluminescence detection with ECL Kit (GE Healthcare, Buenos Aires, Argentina).

Microdilution susceptibility assay

The microdilution susceptibility assay was performed as described by Regenhard *et al*. (2009) with minor modifications. Only *Escherichia coli* strain ATCC 35218 was used for the microdilution assay. In brief, cells were grown for 2 h in Luria-Bertali (LB) medium at 37 °C. Then the bacterial culture was adjusted to 10^4 colony-forming units (CFU)/ml in 10 mM sodium phosphate buffer (pH 7.4).

Aliquots (100 μl) of the microbial suspension were mixed with 10 μl of His6-pS100A7 solution (range of final concentration from 50–150 μg/l) and incubated at 37 °C. After 3 h, the total volume of test suspensions was plated onto LB-Agar. After overnight incubation at 37 °C, CFU were counted. Results are given as CFU/ml. Two-way analysis of variance (ANOVA) was applied and individual means were further tested by least significant difference (LSD) test.

Radial diffusion assay

A radial diffusion assay was performed as in Lehrer *et al*. (1991) with modifications. Bacteria were grown to mid-log phase in LB medium and plated into Petri dishes (10 ml). After plating, a 5 μl aliquot of each protein solution was pipetted into wells formed with a biopsy punch (diameter: 3 mm), and plates were incubated overnight at 37 °C. Ampicillin and kanamycin were used as positive growth inhibition controls.

Phylogeny and sequence analysis of S100A7 proteins in mammals

In order to broaden the study to other mammals, the presence of S100A7 proteins from other mammalian species was sought. The proteins that were manually annotated and reviewed were used for comparison in their annotated form. The proteins that were automatically annotated and not reviewed were compared with the previous ones using a BLAST (Basic Local Alignment Search Tool) (Altschul *et al*., 1997) program to discard redundant data. Accession numbers for S100A7 proteins used in this analysis were: gi|190668 (*Homo sapiens*), gi|114559708 (*Pan troglodytes*), gi|27807077 (*Bos taurus*), gi|110224792 (*Equus caballus*), gi|46397569 (*Mus musculus*), gi|426216655 (*Ovis aries*), EW648856.2 (*Sus scrofa*) and gi|350583366 (*Sus scrofa*). Phylogenetic analyses were carried out using http://www.phylogeny.fr. Multiple sequence alignments were performed using the MUSCLE algorithm. Curation was performed using G-blocks with non-stringent selection parameters. A phylogenetic tree was reconstructed using maximum of likelihood (PhyML) method. Bootstrap values were estimated with 100 replications.

Molecular weight of proteins was estimated from the sequences using bioinformatics tools from http://expasy.org/

https://doi.org/10.1017/S0967199413000464 Published online by Cambridge University Press

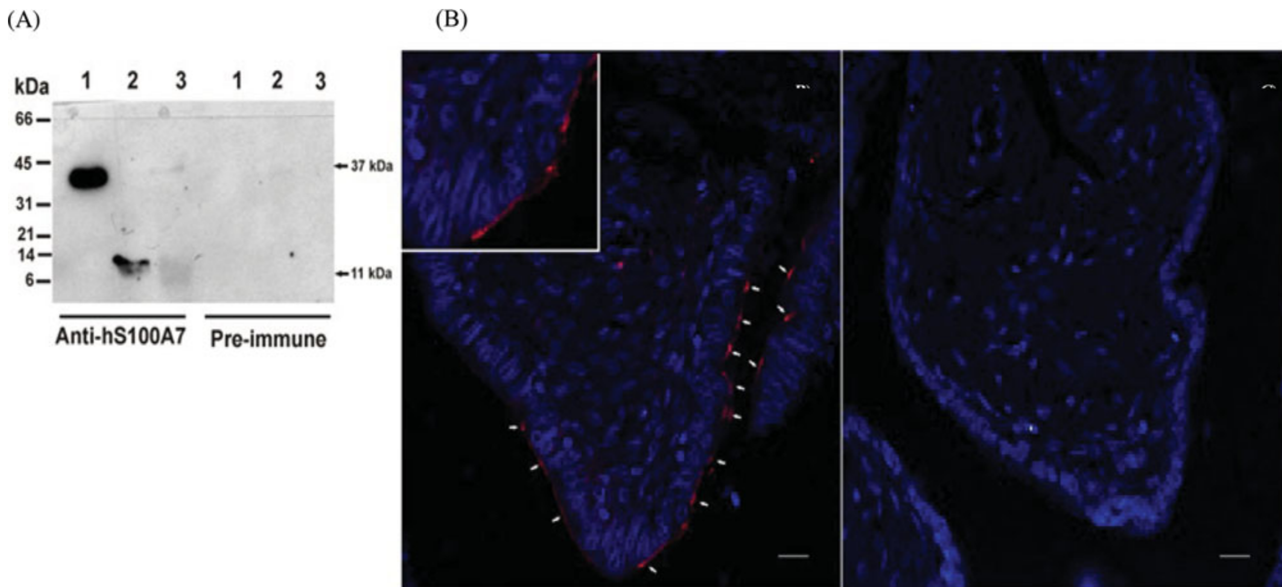

Figure 1 Human S100A7 expression in Fallopian tube and seminal plasma. (*A*) Western blot analyses of Fallopian tube tissue extracts and seminal plasma. Line 1: 2 μg of GST-hS100A7; line 2: Fallopian tube tissue extract (protein content 50 μg); line 3: seminal plasma (protein content 30 μg). Data are representative of six Fallopian tubes and five seminal plasma samples. (*B*) Immunofluorescence showing hS100A7 in the apical cytoplasm of epithelial cells (white arrows). Inset shows ×2.5 magnification. (*C*) Control was performed with pre-immune serum. Nuclei were stained with DAPI. Bar indicates 20 μm.

Results

Presence of S100A7 in Fallopian tubes

To evaluate the presence of S100A7 in Fallopian tubes (human S100A7, hS100A7), tissue protein extracts were subjected to western blot assays. Antibodies generated against recombinant GST-hS100A7 were purified by affinity to recombinant His6-hS100A7 and used for this assay (Teijeiro & Marini, 2012b). Fig. 1*A* shows the presence of hS100A7 in protein extracts from Fallopian tubes and in seminal plasma. Immunofluorescence analysis showed that hS100A7 is located at the apical cytoplasm of Fallopian epithelial cells (Fig. 1*B*, white arrows, left panel). Negative controls were developed with pre-immune serum (Fig. 1*C*. right panel).

Presence of S100A7 in porcine tissues

Porcine S100A7 was initially cloned from tongue RNA (Gorodkin *et al.*, 2007). To corroborate the protein expression in its original tissue resource and to evaluate the quality of the antiserum prepared using His6-pS100A7, protein extracts from this organ were subjected to western blot. As shown in Fig. 2*A*, different amounts of a band of 11 kDa that corresponded to pS100A7 were observed for different quantities of tongue extracts revealed with anti-His6-pS100A7. Pre-immune serum showed no signal. Having checked the quality of anti-His6-pS100A10 antiserum, the presence of pS100A7 in different reproductive tissues was evaluated by western blot. From all the assayed reproductive tissues, only the seminal vesicles showed the presence of pS100A7. Seminal plasma also showed

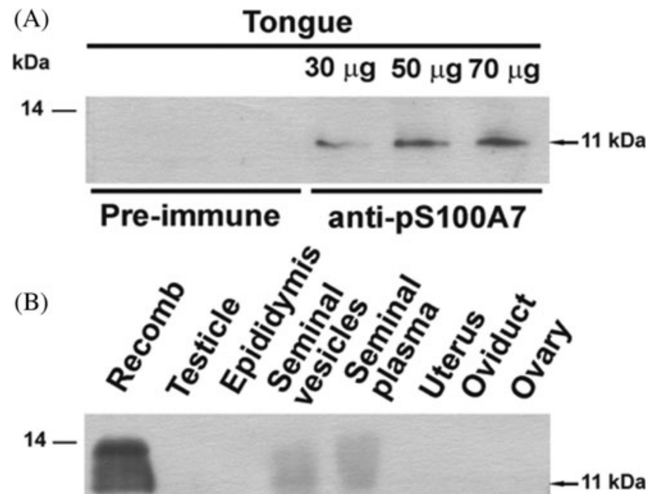

Figure 2 Presence of S100A7 in porcine tissues. (*A*) Expression of pS100A7 in tongue. Different amounts of tissue extracts were assayed by western blot showing increased amounts of pS100A7. Control was performed with pre-immune serum. (*B*) Analysis of expression of pS100A7 in porcine reproductive tissues by western blot. Only seminal vesicles and seminal plasma showed signal. Tissue extracts containing 50 μg of protein were used. Recomb: recombinant pS100A7 (2 μg).

https://doi.org/10.1017/S0967199413000464 Published online by Cambridge University Press

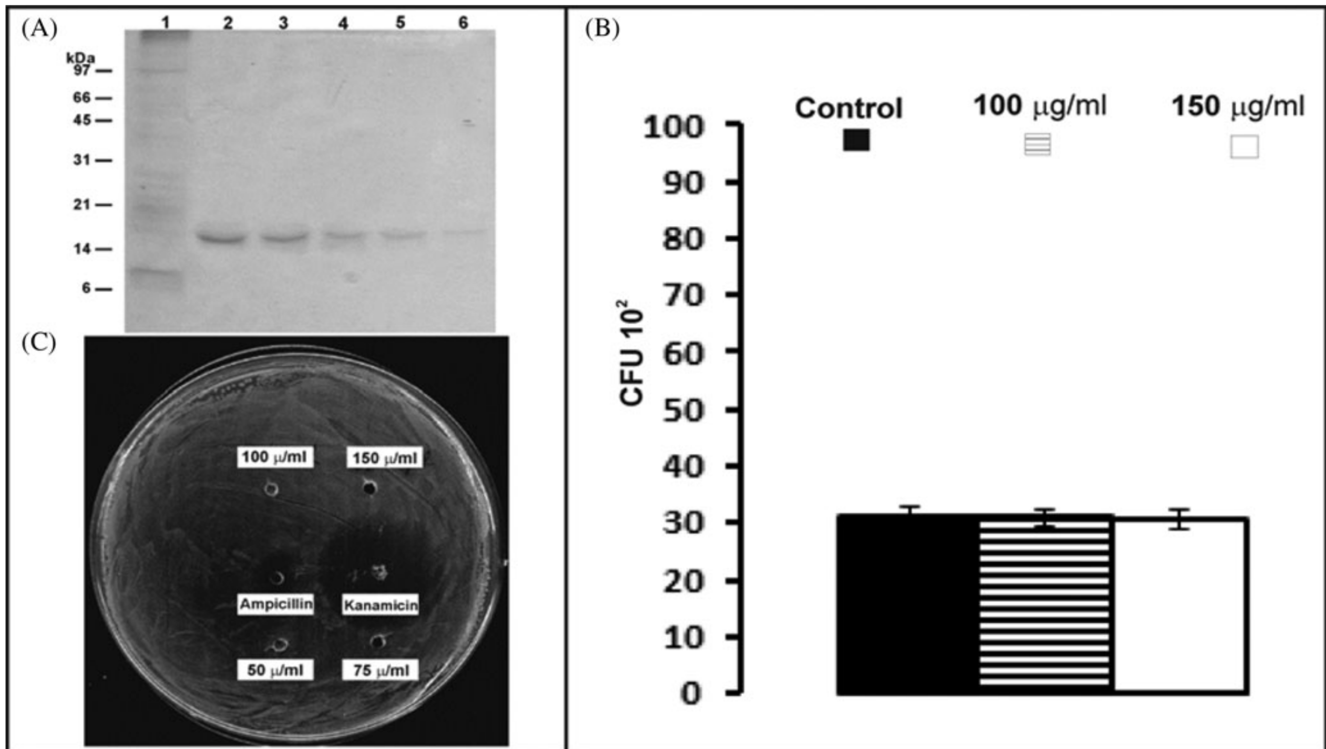

Figure 3 Analyses of His6-pS100A7 anti-microbial activity. (*A*) Purification of recombinant pS100A7: 10 microliter samples from purified aliquots sequentially eluted of His6-pS100A7 were analysed by silver-stained SDS-PAGE (lines 2–6). Line 1: 10 µg *E. coli* BL21 IPTG induced culture extract. (*B*) *E coli* ATCC 35218 was treated with purified His6-pS100A7. Only two of the highest concentrations used are shown. Microdilution susceptibility assay shows no differences in CFU counting between treatments and control. (*C*) Radial diffusion assay shows no clear zone surrounding the wells loaded with different concentrations of His6-pS100A7. Ampicillin and kanamycin were used as positive controls.

signal. S100A7 was not detected on porcine female organs under these conditions.

His6-pS100A7 anti-microbial activity analyses

Previous reports indicate that cow S100A7 and human S100A7 have anti-microbial activity, preferentially against *E. coli*. Sequentially eluted His6-pS100A7 fractions were purified with the efficacy illustrated in Fig. 3*A*, pooled and re-buffered with PBS. Purified His6-pS100A7 was then used for anti-microbial activity assays. Testing the effect on the same *E. coli* strain, ATCC 35218, by microdilution susceptibility and radial diffusion assays, no anti-microbial activity for recombinant pS100A7 could be detected. There was no significant difference between CFU in *E. coli* treated with His6-pS100A7 and controls (Fig. 3*B*). Also, complementary radial diffusion assay showed no clear zones in wells that contained different concentrations of recombinant pS100A7, which indicated no inhibition of *E. coli* growth by pS100A7, in comparison with well known anti-microbial compounds (Fig. 3*C*).

Phylogeny analysis of S100A7 proteins in mammals

A phylogenetic tree was reconstructed using all the sequences indicated in the Materials and methods section. The phylogenetic reconstruction and the multiple alignment data showed that the amino acid sequence of *Mus musculus* (gi | 46397569) and *Sus scrofa* (EW648856.2) S100A7 are highly divergent from the other sequences (Fig. 4*A*, *B*). As would be expected from patterns of mammalian evolution, primate and ruminant S100A7 cluster together. The phylogenetic tree reconstructed using the PhyML method suggests that the equine homologue is more closely related to primate sequences, while porcine and murine proteins form different branches.

Analysis of molecular weights, calculated using bioinformatics tools, showed that the weight for the automatically derived porcine S100A7 sequence (gi | 350583366) was 17621.97 Da, while that for tongue cDNA cloned porcine S100A7 (EW648856.2) was 11653.99 Da (Fig. 4*C*, in bold). The latter was confirmed to be present in tongue tissue by western blot in the current study (Fig. 2*A*). It should be noted that the amino acid at position 99 of the polypeptide derived

https://doi.org/10.1017/S0967199413000464 Published online by Cambridge University Press

(C)

Sus scrofa: gi|350583366

MAIRTSGPRLEGPAPGAAETPHPLQDVKVAVTLLPGPGPAQLTQPPLQGRPLSSAVSKAK**MSTTPAEKSM**
MDTIDLFHKFTDDSDTMDKEGLLKLLQENFPNFLSACDKNGVDYLANIFEQKDKNKDQRIEFSEFLSLLG
DIATGYHKHSHQQELCPPGHQ

Figure 4 Phylogenetic reconstruction and alignment of S100A7 proteins. (*A*) Phylogenetic tree reconstructed by the PhyML method. Branch lengths indicate rates of evolution. (*B*) Alignment of sequences used for the phylogenetic tree reconstruction. (*C*) Sequence of porcine S100A7 predicted by automated computational analysis (gi | 350583366). The sequence of porcine S100A7 derived from the cDNA cloned by Gorodkin and co-workers (2007) is showed in bold.

from tongue cDNA is glutamic acid, while that from the automatically derived sequence is glycine.

Discussion

The mammalian oviduct is the natural site where final gamete maturation, fertilization and early embryo development take place (Harper, 1994). The oviduct is a dynamic organ that modulates gamete's functions and provides a proper environment for early embryo development (Pulkkinen, 1995). In the past, functions have been identified and established for oviductal proteins, such as annexin A2 and DMBT1 (previously called SBG (sperm-binding glycoprotein)) (Teijeiro & Marini, 2012a; Teijeiro *et al.*, 2008, 2009, 2011, 2012), and S100A7 has been identified as a boar sperm protein that interacts with DMBT1 (Teijeiro & Marini, 2012b). Further, S100A7 has been identified and localized on human sperm head (Teijeiro & Marini, 2012b). The current work searched for S100A7 on the female reproductive tract, where it could have important functions in innate immunity.

S100A7 was originally cloned from unfractionated uncultured psoriatic keratinocytes (Madsen *et al.*, 1991) and functions such as transglutaminase substrate/cornified envelope precursor, signal transduction protein, chemokine and anti-bacterial protein in normal epidermis were assigned to it (Eckert & Lee, 2006). The oviductal epithelium is the tissue involved in oviductal fluid secretion, in which gametes and embryo development are supported (Leese *et al.*, 2001). The current results demonstrate that S100A7 is present in the epithelium of Fallopian tubes (Fig. 1). This finding is important because the Fallopian tube is considered to be a 'sterile site' (Quayle, 2002) and an eventual infection in this organ could cause tubal infertility or tubal ectopic pregnancy (Frew & Stock, 2011). It has been demonstrated that human S100A7 has anti-bacterial properties against a spectrum of bacteria (Gläser *et al.*, 2005) and thus hS100A7 may contribute to the 'sterility' of the Fallopian tube. Its presence in human seminal plasma (Fig. 1) and in the sperm head (Teijeiro & Marini, 2012b) may be a product of secretion from the male epithelial reproductive tract and also function on anti-bacterial activity. In pigs, western blot expression analyses allowed the observation of pS100A7 in seminal vesicles and seminal plasma, in accordance with an origin for pS100A7 in seminal vesicles. However, in contrast with human, the protein was not detected in female porcine tissues.

Originally cloned from the tongue (Gorodkin *et al.*, 2007), porcine S100A7 was detected by western blot in tongue tissue extracts, and confirmed the expression of the protein in this tissue (Fig. 2*A*). It is interesting to note that pS100A7 shares localization with hS100A7 in this organ. Unlike hS100A7, no pS100A7 was found

https://doi.org/10.1017/S0967199413000464 Published online by Cambridge University Press

in the oviductal epithelium (Fig. 2*B*). The presence of hS100A7 was detected in human testicles and ovaries (Shadeo *et al*., 2007) but pS100A7 was not detected in the same tissues from pigs. Thus, S100A7 seems to exhibit a conserved role in the tongue that is not observed between human and porcine reproductive tissues.

Anti-microbial activity for His6-pS100A7 was not detected either by microdilution susceptibility assay or by radial diffusion assay (Fig. 3*B*, *C*); however, this activity cannot be ruled out.

As expected from mammalian pattern evolution, the current phylogenetic reconstruction shows that primates and ruminants cluster together (Fig. 4). Although other primate S100A7 sequences are available, for example *Gorilla gorilla* (gi|426331624), they show extremely high homology and thus chimpanzee was chosen as representative of the group. Equine S100A7 is more related to S100A7 from primates, but no information could be found about its functions or localizations. In agreement with Wolf *et al*. (2006), the current phylogenic reconstruction suggests that murine S100A7 may be the ancestral gene for human S100A7 and, moreover, for the rest of the species analysed. Porcine S100A7 forms a different branch from that of most species, and this might be related to the lack of the anti-microbial activity shown, in contrast to the bovine and human proteins. Mutation of the carboxyterminal region of human S100A7 slightly reduces psoriasin's anti-bacterial activity (Lee & Eckert, 2007). In this regard, the lack of homology between the porcine and human proteins at the carboxyterminal end might be responsible for the lack of anti-microbial activity of pS100A7, and indicates that innate immunity may not be the main function of S100A7 in porcine reproductive tracts. Bioinformatic tools derived the molecular weight for the automatically annotated porcine protein (gi|350583366) (Fig. 4) as 17621.97 Da, while that of S100A7 cloned from pig tongues was 11653.99 Da. The tongue-cloned molecular weight is in agreement with the weight of the actual protein observed by western blot. The apparent molecular mass corresponds to the sequence obtained from tongue cDNA instead of the one deduced from the DNA sequence (gi|350583366) (Fig. 4*C*).

Acknowledgements

We thank Dr Sergio Ghersevich for Fallopian tube samples and Dr Claus Jørgensen for pig EST clone. We also thank Frigorífico Palidini S.A. This work was supported in part by the ANPCyT-BID program PICT 01–15092 of Argentina.

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389–402.

Avilés, M., Guitiérrez-Adán, A. & Coy, P. (2010). Oviductal secretions: will they be key factors for the future ARTs? *Mol. Hum. Reprod.* **16**, 896–906.

Demott, R.P. & Suárez, S.S. (1992). Hyperactivated sperm progress in the mouse oviduct. *Biol. Reprod.* **46**, 779–85.

Eckert, R.L. & Lee, K.C. (2006). S100A7 (Psoriasin): a story of mice and men. *J. Invest. Dermatol.* **126**, 1442–4.

Fazeli, A., Elliott, R.M., Duncan, A.E., Moore, A., Watson, P.F. & Holt, W.V. (2003). *In vitro* maintenance of boar sperm viability by a soluble fraction obtained from oviductal apical plasma membrane preparations. *Reproduction* **125**, 509–17.

Flechon, J.E. & Hunter, R.H. (1981). Distribution of spermatozoa in the utero-tubal junction and isthmus of pigs, and their relationship with the luminal epithelium after mating: a scanning electron microscope study. *Tissue & Cell* **13**, 127–39.

Frew, L. & Stock, S.J. (2011). Antimicrobial peptides and pregnancy. *Reproduction* **141**, 725–35.

Gläser, R., Harder, J., Lange, H., Bartels, J., Christophers, E. & Schröder, J.M. (2005). Antimicrobial psoriasin (S100A7) protects human skin from *Escherichia coli* infection. *Nat. Immunol.* **6**, 57–64.

Gorodkin, J., Cirera, S., Hedegaard, J., Gilchrist, M.J., Panitz, F., Jørgensen, C., Scheibye-Knudsen, K., Arvin, T., Lumholdt, S., Sawera, M., Green, T., Nielsen, B.J., Havgaard, J.H., Rosenkilde, C., Wang, J., Li, H., Li, R., Liu, B., Hu, S., Dong, W., Li, W., Yu, J., Wang, J., Staefeldt, H-H., Wernersson, R., Madsen, L.B., Thomsen, B., Hornshøj, H., Bujie, Z., Wang, X., Wang, X., Bolund, L., Brunak, S., Yang, H., Bendixen, C. & Fredholm, M. (2007). Porcine transcriptome analysis based on 97 non-normalized cDNA libraries and assembly of 1,021,891 expressed sequence tags. *Genome Biol*. **8**, R45. doi:10.1186/gb-2007–8-4-r45º

Harper, M.J.K. (1994). Gamete and zygote transport. In *The Physiology of Reproduction* (eds E. Knobil & J.D. Neill), pp.123–87. New York: Raven Press.

Holt, W.V. & Fazeli, A. (2010). The oviduct as a complex mediator of mammalian sperm function and selection. *Mol. Reprod. Dev.* **77**, 934–43.

Hung, P.H. & Suárez, S.S. (2010). Regulation of sperm storage and movement in the ruminant oviduct. *Soc. Reprod. Fertil. Suppl.* **67**, 257–66.

Hunter, R.H. (1981). Sperm transport and reservoirs in the pig oviduct in relation to the time of ovulation. *J. Reprod. Fertil.* **63**, 109–17

Hunter, R.H. (2005). The Fallopian tubes in domestic mammals: how vital is their physiological activity? *Reprod. Nutr. Dev.* **45**, 281–90.

Hunter, R.H.F. & Nichol, R. (1983). Transport of spermatozoa in the sheep oviduct: preovulatory sequestering of cells in the caudal isthmus. *J. Exp. Zool.* **228**, 121–8.

Hunter, R.H. & Wilmut, I. (1984). Sperm transport in the cow: peri-ovulatory redistribution of viable cells within the oviduct. *Reprod. Nutr. Dev.* **24**, 597–608.

https://doi.org/10.1017/S0967199413000464 Published online by Cambridge University Press

Ignotz, G.G., Cho, M.Y. & Suárez, S.S. (2007). Annexins are candidate oviductal receptors for bovine sperm surface proteins and thus may serve to hold bovine sperm in the oviductal reservoir. *Biol. Reprod.* **77**, 906–13.

Killian, G. (2011). Physiology and endocrinology symposium: evidence that oviduct secretions influence sperm function: a retrospective view for livestock. *J. Anim. Sci.* **89**, 1315–22.

Lee, K.C. & Eckert, R.L. (2007). S100A7 (Psoriasin)–mechanism of antibacterial action in wounds. *J. Invest. Dermatol.* **127**, 945–57.

Leese, H.J., Tay, J.I., Reischl, J. & Downing, S.J. (2001). Formation of Fallopian tubal fluid: role of a neglected epithelium. *Reproduction* **121**, 339–46.

Lehrer, R.I., Rosenman, M., Harwig, S.S.S.L. & Eisenhauer, P. (1991). Ultrasensitive assays for endogenous antimicrobial polypeptides. *J. Immunol. Methods* **137**, 167–73.

Madsen, P., Rasmussen, H.H., Leffers, H., Honore, B., Dejgaard, K., Olsen, E., Kiil, J., Walbum, E., Andersen, A.H., Basse, B., Lauridsen, J.B., Ratz, G.P., Celis, A., Vandekerckhove, J. & Celis, J.E. (1991). Molecular cloning, occurrence, and expression of a novel partially secreted protein "psoriasin" that is highly up-regulated in psoriatic skin. *J. Invest. Dermatol.* **97**, 701–12.

Mildner, M., Stichenwirth, M., Abtin, A., Eckhart, L., Sam, C., Gläser, R., Schröder, J-M., Gmeiner, R., Mlitz, V., Pammer, J., Geusau, A. & Tschachler, E. (2010). Psoriasin (S100A7) is a major *Escherichia coli*-cidal factor of the female genital tract. *Mucosal Immunol.* **3**, 602–9.

Pacey, A.A., Davies, N., Warren, M.A., Barratt, C.L. & Cooke, I.D. (1995). Hyperactivation may assist human spermatozoa to detach from intimate association with the endosalpinx. *Hum. Reprod.* **10**, 2603–9.

Pérez, F.A., Roma, S.M., Cabada, M.O. & Marini, P.E. (2006). Sperm binding glycoprotein is differentially present surrounding the lumen of isthmus and ampulla of the pig's oviduct. *Anat. Embryol.* **211**, 619–24.

Pulkkinen, M.O. (1995). Oviductal function is critical for very early human life. *Ann. Med.* **27**, 307–10.

Quayle, A.J. (2002). The innate and early immune response to pathogen challenge in the female genital tract and the pivotal role of epithelial cells. *J. Reprod. Immunol.* **57**, 61–79.

Rabilloud, T., Vuillard, L., Gilly, C. & Lawrence, J.J. (1994). Silver-staining of proteins in polyacrilamide gels: a general overview. *Cell. Mol. Biol. (Noisy-le-grand)* **40**, 57–75.

Regenhard, P., Leippe, M., Schubert, S., Podschun, R., Kalm, E., Grötzinger, J. & Looft, C. (2009). Antimicrobial activity of bovine psoriasin. *Vet. Microbiol.* **136**, 335–40.

Shadeo, A., Chari, R., Vatcher, G., Campbell, J., Lonergan, K.M., Matisic, J., van Niekerk, D., Ehlen, T., Miller, D., Follen, M., Lam, W.L. & MacAulay, C. (2007). Comprehensive serial analysis of gene expression of the cervical transcriptome. *BMC Genomics* **8**, 142. DOI: 10.1186/1471–2164–8-142

Suárez, S.S. (1998). The oviductal sperm reservoir in mammals: mechanisms of formation. *Biol. Reprod.* **58**, 1105–7.

Suárez, S.S. (2007). Interactions of spermatozoa with the female reproductive tract: inspiration for assisted reproduction. *Reprod. Fert. Dev.* **19**, 103–10.

Suárez, S.S. (2008). Regulation of sperm storage and movement in the mammalian oviduct. *Int. J. Dev. Biol.* **52**, 455–62.

Teijeiro, J.M. & Marini, P.E. (2012a). The effect of oviductal deleted in malignant brain tumor 1 over porcine sperm is mediated by a signal transduction pathway that involves pro-AKAP4 phosphorylation. *Reproduction* **143**, 773–85.

Teijeiro, J.M. & Marini, P.E. (2012b). S100A7 is present in human sperm and a homologous pig sperm protein interacts with sperm-binding glycoprotein (SBG). *Andrologia* **44 (Suppl 1)**, 772–9.

Teijeiro, J.M, Cabada, M.O. & Marini, P.E. (2008). Sperm binding glycoprotein (SBG) produces calcium and bicarbonate dependent alteration of acrosome morphology and protein tyrosine phosphorylation on boar sperm. *J. Cell. Biochem.* **103**, 1413–23.

Teijeiro, J.M., Ignotz, G.G. & Marini, P.E. (2009). Annexin A2 is involved in pig (*Sus scrofa*) sperm-oviduct interaction. *Mol. Reprod. Dev.* **76**, 334–41.

Teijeiro, J.M., Dapino, D.G. & Marini, P.E. (2011). Porcine oviduct sperm binding glycoprotein and its deleterious effect on sperm: a mechanism for negative selection of sperm? *Biol. Res.* **44**, 329–37.

Teijeiro, J.M., Roldan, M.L. & Marini, P.E. (2012). Molecular identification of the sperm selection involved porcine sperm binding glycoprotein (SBG) as deleted in malignant brain tumors 1 (DMBT1). *Biochimie* **94**, 263–7.

Thomas, P.G., Ball, B.A. & Brinsko, S.P. (1994). Interaction of equine spermatozoa with oviduct epithelial cell explants is affected by estrous cycle and anatomic origin of explant. *Biol. Reprod.* **51**, 222–8.

Wolf, R., Voscopoulos, C.J., FitzGerald, P.C., Goldsmith, P., Cataisson, C., Gunsior, M., Walz, M., Ruzicka, T. & Yuspa, S.H. (2006). The mouse S100A15 ortholog parallels genomic organization, structure, gene expression, and protein-processing pattern of the human S100A7/A15 subfamily during epidermal maturation. *J. Invest. Dermatol.* **126**, 1600–8.

Zumoffen, C.M., Gil, R., Caille, A.M., Morente, C., Munuce, M.J. & Ghersevich, S.A. (2013). A protein isolated from human oviductal tissue in vitro secretion, identified as human lactoferrin, interacts with spermatozoa and oocytes and modulates gamete interaction. *Hum. Reprod.* **28**, 1297–308.

https://doi.org/10.1017/S0967199413000464 Published online by Cambridge University Press