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Immunohistochemical Localization of Sperm-Preserving Proteins in the Ram Reproductive Tract

MARTA FERNÁNDEZ-JUAN,*‡ MARGARITA GALLEGO,† BEATRIZ BARRIOS,*‡ JESÚS OSADA,*‡ JOSÉ A. CEBRIÁN-PÉREZ,*‡ AND TERESA MUIÑO-BLANCO*‡

*From the Departments of *Biochemistry and Molecular and Cell Biology and †Animal Pathology, School of Veterinary Medicine, University of Zaragoza, Zaragoza, Spain.*

ABSTRACT: Previously, we reported that the addition of seminal plasma proteins before cold-shock treatment prevents sperm membrane injury, and that 2 proteins of approximately 14 (P14) and 20 (P20) kDa, the main components of fraction 6 isolated by exclusion chromatography, are responsible for this protective effect. The objective of the present study was to localize P14 and P20 in tissues of the ram reproductive tract to determine their origin. Antiserum generated against purified P14 and P20 reacted with proteins in seminal vesicles and vas deferens by Western blot analyses of protein tissue extracts. However, these antisera failed to detect P14 and P20 in testis, prostate, efferent ductules, bulbourethral glands, and epididymis (caput, corpus, and cauda). Immunohistochemical analyses by both indirect immunofluorescence and the avidin-biotin complex technique confirmed that only seminal vesicles showed reactivity, restricted to the secretory cells, with both antibodies. Obtained results indicate that P14 and P20 are secreted specifically in the seminal vesicles. To further confirm that P14 and P20 are specifically expressed in seminal vesicles, we used Northern blot analyses to investigate the expression of both proteins in seminal vesicles and vas deferens. These assays corroborated again that P14 and P20 were specifically expressed in seminal vesicles. Consequently, we suggest referring to these 2 proteins as RSVP14 and RSVP20, respectively, according to their origin and molecular weight.

Key words: Seminal plasma proteins, seminal vesicles, cold shock, ram spermatozoa.

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Seminal plasma of mammals is composed of secretions from several glands of the male reproductive tract (Mann and Lutwak-Mann, 1981) and contains various proteins, some of which are able to bind to the sperm plasma membrane. Most of these proteins are secretory products of the seminal vesicle (Aumüller et al, 1988; Chandonnet et al, 1990; Dostalova et al, 1995), an accessory reproductive gland in most male mammals. The seminal vesicle secretion (SVS) accumulates in the lumen of this reproductive gland after puberty. This fluid constitutes a portion of seminal plasma on ejaculation and influences the metabolism, motility, and surface properties of spermatozoa (Manco and Abrescia, 1988; Metafora et al, 1988; Peitz, 1988). A precursor molecule of a sperm motility inhibitor in human seminal plasma is synthesized in the seminal vesicles (Robert and Gagnon, 1994). Mammalian spermatozoa have been reported to bind seminal vesicle proteins (Carballada and Esponda, 1998). However, contradictory results about the effect of this binding have been shown, such as inducing capacitation of rat sperm (Manco and Abrescia, 1988; Metafora et al, 1988; Peitz 1988) and suppressing capacitation of mouse sperm (Huang et al, 2000). Furthermore, the removal of the seminal vesicles of several species induced complete infertility (Davis and Niwa, 1974; Queen et al, 1981; Peitz and Olds-Clarke, 1986), greatly decreased the pregnancy rate and litter size of mouse (Pang et al, 1979), and induced a decreased motility of uterine sperm and pregnancy rate (Peitz and Olds-Clarke, 1986). These results indicate the important role of SVS in mammalian sperm function. Finding the structure and function of the seminal vesicle proteins is a prerequisite in understanding their roles in seminal vesicle physiology and their effects on the gamete function.

We recently reported the biochemical characterization and partial amino acid sequence of 2 ram seminal plasma proteins of approximately 14 (P14) and 20 (P20) kDa, which protect spermatozoa against cold shock (Barrios et al, 2005). The P14 sequenced fragment contained the fibronectin domain type II (FN2) domain (Greube et al, 2001), as with bovine PDC-109 (Esch et

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Correspondence to: Dr T. Muiño-Blanco, Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Veterinaria, C/ Miguel Servet, 177, 50013 Zaragoza, Spain (e-mail: muino@unizar.es).

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‡ These authors contributed equally to this article and share senior co-authorship.

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al, 1983), also called bull seminal plasma proteins (BSP) A1/A2 (Manjunath and Sairam, 1987). FN2 domain is a collagen-binding domain that binds to different extracellular matrix and cytoskeleton components to stabilize the extracellular matrix and determine the shape of the cell and cytoskeleton organization. However, the sequence of the P20 fragment was not homologous with any reported protein. The results of immunocytochemical detection and Western blotting analysis of P14 and P20 content on sperm before and after in vitro induction of capacitation and acrosome reaction confirm that the induced membrane alterations account for a decrease in the content as well as migration and redistribution of both proteins to the equatorial and postequatorial regions. Our results suggest that the protective effect of these proteins could be related to their decapacitating role (Barrios et al, 2005).

The aim of the present work was to investigate the expression of P14 and P20 in the ram reproductive tract tissues to determine their origin.

Materials and Methods

Tissue Samples

Tissue samples from testis, prostate, seminal vesicles, vas deferens (the distal fraction including the ampullae), efferent ductules, bulbourethral glands, and epididymis (caput, corpus, and cauda) were collected from 2 freshly slaughtered rams. Tissues were excised, rinsed in phosphate-buffered saline (PBS) pH 7.4, and immediately frozen in liquid nitrogen and stored at −80°C.

Extraction of Proteins from Ram Reproductive Tract Tissues

Proteins from ram reproductive tract tissues were extracted by homogenizing 1–2 g with 1 mL 10 mM sodium phosphate buffer containing 10% of a protease inhibitor cocktail (Sigma Chemical Co, St Louis, Mo) in an ice bath. After centrifuging at 5000 × *g* for 10 minutes at 4°C, the supernatant was recovered and stored at −20°C. Protein concentration was determined according to the method described by Bradford (1976).

Polyclonal Antibodies

Polyclonal antibodies were raised against the whole seminal plasma fraction 6 (F6) isolated by exclusion chromatography in Sephacryl-100 (Barrios et al, 2000), and the purified P14 and P20 bands were recovered by electroelution from a nondenaturing gel (Barrios et al, 2000) by rabbit immunization with 500 μg (F6) and 300 μg (P14 and P20) of protein in Freunds complete adjuvant. After 15 days they were reimmunized with the same amount dissolved in Freunds incomplete adjuvant. The antiserum was obtained 15 days after the second immunization by centrifugation of 10–20 mL of blood from each rabbit and purification by protein G affinity chromatography.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Western Blotting

Immunoblots were carried out by a 7%–22% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of 25- to 100-μg proteins transferred for 2 hours onto a polyvinyldene difluoride membrane with a Hoefer TE70 Semiphor Semidry-Transfer Unit (Pharmacia-Biotech, Uppsala, Sweden). Nonspecific sites on the membranes were blocked for 1 hour with 5% bovine serum albumin (BSA) in blocking buffer (Tris-HCl 10 mM pH 8; NaCl 120 mM, 0.05% Tween 20). The proteins were immunodetected by incubating for 3 hours at 20°C with the polyclonal antibodies diluted at 1:2000 (P14) and 1 : 500 (P20) in blocking buffer that contained 0.17% BSA. After exhaustive washing, the blots were incubated with a secondary goat anti-rabbit alkaline-phosphatase–conjugated IgG (Sigma) at a dilution 1:30 000 for 2 hours. After 4 washings of 5 minutes each, the membranes were incubated with 66 μg/mL 5-bromo-4-chloro-3-indolyl phosphate and 111 μg/mL nitro blue tetrazolium in Tris 0.19 M, $MgCl_2$ 1 mM until color appeared. The image was scanned and a densitometric analysis was carried out. The Gel Doc System with Molecular Analyst software (Bio Rad, Hercules, Calif) was used to quantify the changes in intensity of various bands. Replacing the antiserum with preimmune serum was used as a negative control to rule out nonspecific binding to the transferred proteins.

Indirect Immunofluorescence

Tissue sections (7 μm thick) were cut on a Surgipath microtome (American Instrument Exchange, Inc, Haverhill, Mass), air dried, and fixed in acetone for 20 minutes. Nonspecific binding sites were blocked with 5% BSA in PBS for 30 minutes at room temperature in a humidified chamber. After blocking, the slides were washed 3 times with PBS, and 50 μL of primary polyclonal antibodies diluted in PBS with 1% BSA at 1:250 (P14) and 1:150 (P20) were added. The preparations were incubated for 1 hour at 37°C in the wet chamber, washed 3 times, and incubated again with 250 μL of anti-rabbit IgG (Alexa Fluor 488, Molecular Probes (Leiden, The Netherlands); 1:900 diluted in PBS with 1% BSA) for 1½ hours at 37°C in the dark. The slides were rinsed with PBS. Finally, the preparations were covered with coverslips, sealed with colorless enamel, and visualized through a Nikon Eclipse E-400 microscope (Tokyo, Japan) under epifluorescence illumination with a fluorescein filter at 10× and 40×. Negative controls were performed by omission of the primary antibodies and also by replacing the primary antibody with normal rabbit serum.

Immunohistochemical Techniques

Tissue samples were fixed in Bouins solution for 90 minutes and dehydrated in a graded series of ethanol and embedded in paraffin. Sections (7 um thick) were cut on a Surgipath

*Oligonucleotide sequences used for polymerase chain reaction amplifications and sequencing**

P14-5′	5′-GAT GAT GAI CTI ACI CG-3′
P20-5′	5′-GAT GAI CCI CTI CCI GA-3′
Left seminal vesicle	5′-GCT GGT GCT CAA GTG GAG A-3′
Right seminal vesicle	5′-CTG CCA AGC ATA TTC TCG CT-3′

* Oligonucleotide primers were synthesized at Sigma-Genosys Ltd (Haverhill, United Kingdom). I indicates inosine.

microtome. The sections were exposed to immunohistochemical staining by an avidin-biotin complex technique (Vector, Burlingame, Calif). Endogenous peroxidase was inactivated with 1.7% hydrogen peroxide (H_2O_2) in 100% ethanol for 30 minutes. Subsequently, the sections were washed in PBS pH 7.4 and then incubated with normal goat serum (blocking reagent) (Vector) for 20 minutes, followed by incubation with the specific antiserum (primary antibody, dilution 1:125 for P14 and 1:200 for P20) overnight at room temperature. After washing, the slides were incubated with biotinylated anti-rabbit antiserum (Vector) for 30 minutes. An avidin-biotin-peroxidase complex (Vector) was then applied for 40 minutes. The binding sites of the primary antibodies were visualized by diaminobenzidine (DAB) and H_2O_2 solution (0.12 g DAB in 240 mL of PBS pH 7.4 containing 3 % H_2O_2) for 7 minutes. Slides were contrasted with Carazzi hematoxylin and mounted with 1,3 diethyl-8-phenylxanthine. As a negative control, samples were incubated with normal goat serum instead of the primary antibody, with the remaining procedure being the same.

The images obtained by these techniques were taken with a microscope digital camera system (Sony 2CCd Colour Video Camera, and Sony Digital Still Recorder, Carson, Calif) and saved and edited with Visilog 5.1 Software (Noesis S.A, Orsay, France).

RNA Isolation and Reverse Transcriptase–Polymerase Chain Reaction

Total RNA was extracted from seminal vesicles and vas deferens by the guanidinium thiocyanate/phenol extraction method (Chomczynski and Sacchi, 1987; Chomczynski, 1993) by homogenization in 1 mL of TRI REAGENT (Sigma) per 200 mg of tissue. RNA concentration was estimated by reading the absorbance at 260 nm and checked for purity at 280 nm on a Hitachi U-2001 spectrophotometer (Lexington, Ky). Samples were stored at −80°C until used.

Between 200 and 250 ng of total RNA from seminal vesicles was reverse transcribed by using poly (dT) primer and the SuperScript III RT enzyme (Invitrogen Inc, Carlsbad, Calif). One tenth of reverse-transcribed sample was used in the polymerase chain reaction (PCR) amplifications (SuperScript III First-Strand Synthesis System for RT-PCR, Invitrogen Inc).

Amplification of Partial cDNA Sequences Encoding P14 and P20

Degenerate PCR primers (see Table) for P14 and P20 were designed on the basis of the primary sequence of the proteins (Barrios et al, 2005) in the 5′ region. Primers P14-5′ and P20-5′ were based on amino acids residues 1–6. The 3′ primer in both cases was Oligo $(dT)_{20}$. Another primer pair left/right (LP/RP, respectively) was designed by Primer3 Software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

PCR was performed with 2 μL of cDNA. Using these primers, 35 PCR cycles were carried out on reverse-transcribed RNA from ram seminal vesicles. Cycling conditions consisted of 45-second denaturation at 94°C, 1-minute and 30-second annealing (57°C for P14-5′, 53°C for P20-5′, and 66°C for LP/RP), and 3-minute primer extension at 72°C. A 1-minute denaturation step at 94°C preceded cycling; at the end, a final 10-minute primer extension at 72°C was performed, followed by a 24-hour soak at 4°C. The "hot start" procedure was accomplished by adding 1 U of Platinum *Taq* DNA polymerase high fidelity (Invitrogen Inc) to each reaction tube.

cDNA Sequences

PCR fragments covering the complete P14 and P20 cDNA were sequenced. This was performed on both strands with either LP/RP for P20 or P14-5′/Oligo $(dT)_{20}$ primers for P14. DNA was sequenced and analyzed on an ABI Prism 3700 sequencer (Applied Biosystems, Foster City, Calif).

PCR products were separated on 1% agarose gel in 1 × Tris-borate-EDTA buffer containing 0.4 ng/μL ethidium bromide (Sigma-Aldrich) and were visualized under ultraviolet (UV) light. The intensity of each band was assessed by densitometry using an image analysis program (Molecular Analyst software, Bio Rad). Molecular size was stimated by using Step Ladder 50 bp (Sigma).

PCR products were purified by using the GENECLEAN Turbo Nucleic Acid Purification kit (Q-BIO gene, Morgan Irvine, Calif) according to the manufacturer's instructions.

Northern Blot Analysis

Five micrograms of total RNA were denatured at 75°C for 15 minutes and electrophoresed in a 1.2% agarose gel containing 7% formaldehyde. After electrophoresis, the RNAs were transferred to a nylon membrane (Hybond-N, Amersham, Arlington Heights, Ill) for at least 18 hours. The membrane was hybridized in a solution consisting of 50% formamide, 3 × SSC (0.45 M NaCl, 45 mM sodium citrate), 1 × Denhardts solution, 5% dextran sulphate, 2% SDS, 20 μg/mL denatured salmon sperm DNA, and ^{32}P-labeded DNA probes at 42°C. The membrane was washed twice in 3 × SSC and 0.5% SDS at room temperature and twice in 3 × SSC and 0.5% SDS at 50°C for 60 minutes. After drying, the membrane was cross-linked by exposure to UV light.

The P20 and P14 probes were synthesized by reverse transcriptase–PCR (RT-PCR) from seminal vesicle total

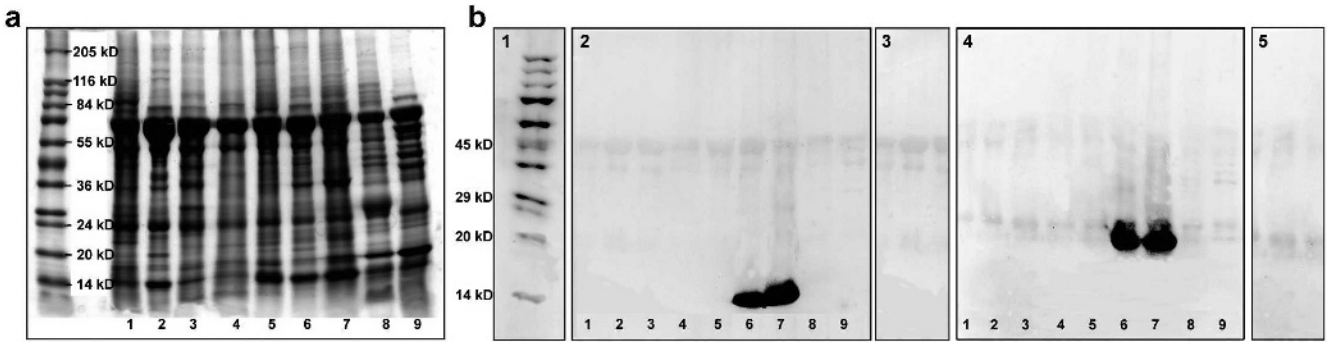

Figure 1. Western blot detection of P14 and P20 in protein extracted from testis (1), efferent ductules (2), caput of epididymis (3), corpus of epididymis (4), cauda of epididymis (5), vas deferens (6), seminal vesicles (7), bulbourethral gland (8), and prostate (9). **(a)** Coomassie blue-stained proteins after sodium dodecyl sulfate–polyacrylamide gel electrophoresis separation. **(b)** 1: Molecular weight markers. 2: Western blot detection of P14. 3: Negative control. 4: Western blot detection of P20. 5: Negative control.

RNA. A mouse 18S probe obtained from plasmid pUC 830 (752-bp EcoRI/BamHI fragment) was used to normalize the amount of RNA loaded on the gel. Both probes were labelled using [α-^{32}P]-dCTP and Rediprime (Amersham). Filters were exposed to Biomax film (Kodak, Amersham) and analyzed by Molecular Analyst software (Bio Rad).

Results

Western Blot Analyses of P14 and P20 in Protein Extracts From the Ram Reproductive Tract Tissues

We have recently shown that 1 protein fraction separated from seminal plasma by exclusion chromatography on Sephacryl-100 HR (F6) is able to prevent cold-shock membrane damage, and that 2 protein bands of this fraction of approximately 14 (P14) and 20 (P20) kDa are responsible for this protective effect (Barrios et al, 2005). Western blot analyses of protein extracted from testis, prostate, seminal vesicle, vas deferens (the distal fraction including the ampullae), efferent ductules, bulbourethral gland, and epididymis (caput, corpus, and cauda) separated by SDS-PAGE, probed with antibodies raised against P14 or P20 recovered by electroelution from a nondenaturing gel, showed that only the extracts from seminal vesicles and vas deferens detected 2 protein bands at 14 kDa (Figure 1b, panel 2, lanes 6 and 7) and 20 kDa (Figure 1b, panel 4, lanes 6 and 7). These results were highly specific because replacing the antiserum with preimmune serum nullified protein detection (Figure 1b, panels 3 and 5).

Indirect Immunofluorescence Analysis

To discard the possibility that the vas deferens signaling was due to contamination from the seminal vesicle while obtaining the sample, we used immunofluorescence to determine the presence of both proteins on slides of seminal vesicle, vas deferens, and corpus and cauda of epididymis. Only the seminal vesicle sample showed fluorescence labeling, restricted to the secretory cells, with the antibodies raised against P14 (Figure 2a) or P20 (Figure 2b). Immunofluorescence of vas deferens and corpus and cauda of epididymis samples was negative with both antisera (anti-P14 or anti-P20) (data not shown). Replacing the antiserum with preimmune serum abolished fluorescence detection (data not shown).

Immunohistochemical Localization by Avidin-Biotin Complex Technique

Because the high intensity of the fluorescent signaling impeded our deduction of just where the protein synthesis takes place, we performed immunohistochemical analyses by the avidin-biotin complex technique. The obtained results confirmed that only the seminal vesicles synthesize P14 and P20, as a characteristic labeling pattern for immunoreactive proteins was found only in this gland. In all other tissue samples, immunoreactivity was negative with both antisera used (P14 or P20). Figure 3 shows the absence of labeling in vas deferens with anti-P14 (Figure 3a) or anti-P20 (Figure 3b) antibodies.

Detectable immunostaining was only found in seminal vesicles. With the P14 antiserum (Figure 4a and c), we found that the reactivity was restricted to the secretory cells of the seminal vesicle epithelium with an intense immunoreaction over the apical plasma membrane, though not all secretory cells showed intracellular staining. Likewise, the apical end of some secretory cells formed protrusions, which dilate and pinch off after selective accumulation of the positive secretory product. This manner of releasing secretion could be regarded as an apocrine-like secretion mechanism.

Similarly, not all the secretory cells showed intracellular staining with the P20 antiserum (Figure 4b and d). In specific regions, most cells were secreting proteins

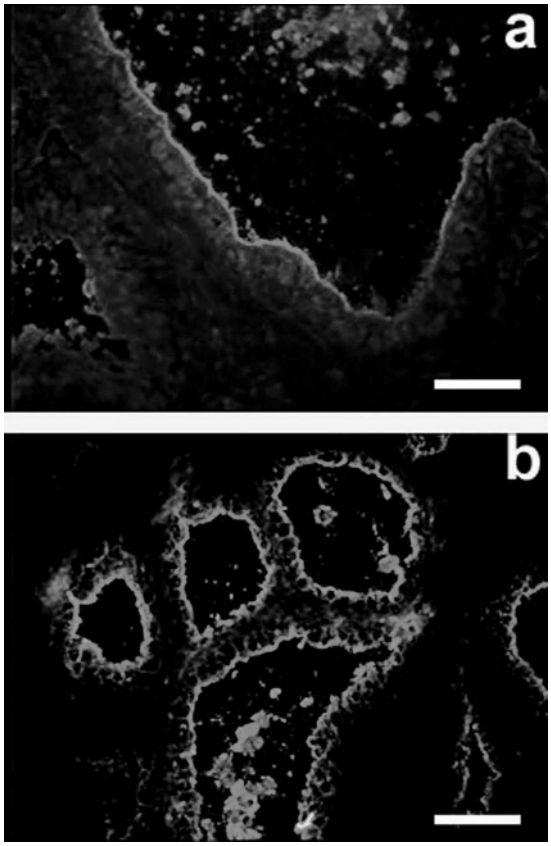

Figure 2. Indirect immunofluorescence localization of P14 and P20 in the seminal vesicle slides with anti-P14 **(a)** or anti-P20 antibodies **(b)**. Bar: a = 0.05 μm; b = 0.1 μm.

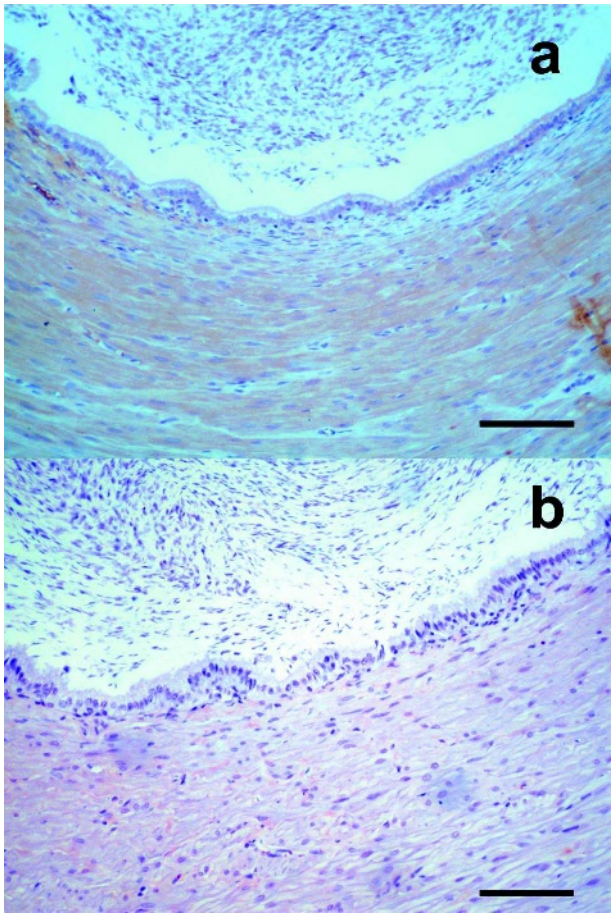

Figure 3. Immunohistochemical localization of P14 and P20 in the vas deferens (absence of labeling) with anti-P14 **(a)** or anti-P20 **(b)** antibodies. Bar: a, b = 100 μm.

with a marked reactivity over the apical membrane and within the cytoplasm. Likewise, apical blebs from the apical cytoplasm of certain cells protrude toward the lumen, with their eventual detachment and breakdown in the lumen.

P14 and P20 Expression Analyzed by RT-PCR and Northern Blot

To further confirm that P14 and P20 are specifically expressed in seminal vesicles, we used Northern blot analyses to investigate the expression of both proteins in both seminal vesicles and vas deferens.

P20 and P14 were first analyzed by RT-PCR with the primer pairs deduced from the analyzed peptide sequence. Specific primers (P14-5′ for P14 and P20-5′ for P20) were designed according to our previous results (Barrios et al, 2005) and their paired primers were Oligo$(dT)_{20}$. When the RT-PCR products were analyzed by 1% agarose gel electrophoresis, a single band of approximately 450 bp (P14) and 500 bp (P20) (assessed as indicated in ''Materials and Methods'') was amplified (data not shown). Both target amplicons were absent in the RT-PCR products from vas deferens samples (data not shown).

Northern blot analysis of total RNAs isolated from seminal vesicles and vas deferens of the adult ram was carried out with the 300-bp fragment of P20 (obtained from specific primers left/right, synthesized by Primer3 Software, on the basis of nucleotide sequence of the former RT-PCR product) and the 450-bp fragment of P14 cDNA as probes. We found a 515-bp mRNA signal for P14 (Figure 5a) and a 600-bp mRNA signal for P20 (Figure 5b) in the seminal vesicles. No signal was found in the vas deferens (Figure 5). These results indicate that the positive reaction with the antibody found in the vas deferens (Figure 1) could be because of contamination from the seminal vesicles during collection of the tissues or because of the presence of spermatozoa in the lumen (Figure 3), as we have already shown that P14 and P20 are adsorbed onto the sperm surface (Barrios et al, 2005).

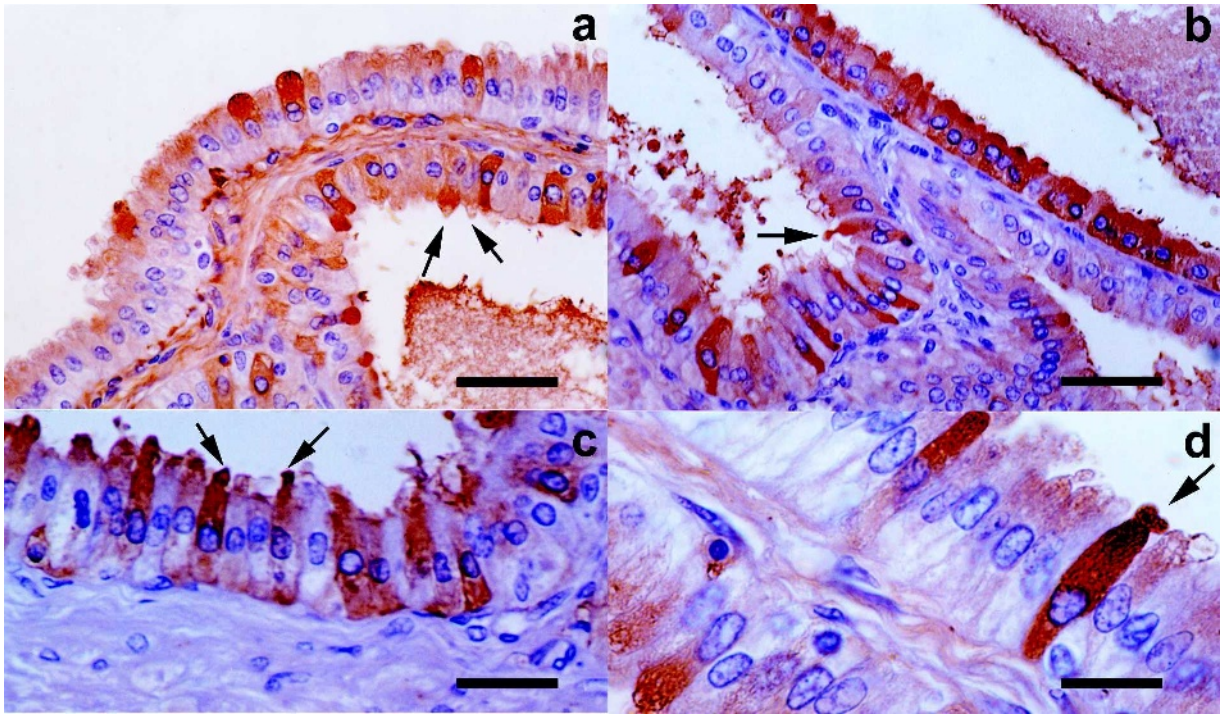

Figure 4. Immunohistochemical localization of P14 **(a, c)** and P20 **(b, d)** in the ram seminal vesicle slides with anti-P14 or anti-P20 antibodies, respectively. Not all secretory cells show intracellular staining. Arrows: The apical end of some secretory cells formed protrusions, which dilate and pinch off after selective accumulation of the positive secretory product. Bar: a = 0.017 μm; b = 0.01 μm; c = 0.015 μm; d = 0.007 μm.

Discussion

Previously, we demonstrated that the adsorption of seminal plasma proteins on ram sperm plasma membranes could partially prevent and repair cold-shock sperm membrane damage (Pérez-Pé et al, 2001a,b), and that this adsorption modified the functional characteristics of damaged spermatozoa reproducing those of live cells (Barrios et al, 2000; Pérez-Pé et al, 2001a). More recently (Barrios et al, 2005), we have proven that 2 proteins of approximately 14 (P14) and 20 (P20) kDa are responsible for this protective and restoring effect. By cytochemical analysis, we showed that both proteins have several binding sites on the sperm surface, and the NH_2-terminal sequencing showed that the P14 fragment has a high homology with the bovine PDC-109 (Esch et al, 1983), GSP-14/15 kDa (goat seminal plasma protein, related to the BSP family) (Villemure et al, 2003), and the recently reported RSP15 of ram (Bergeron et al, 2005). The homology was found from amino acid 19–34 (CVFPFTYYDDRHFDCT) with 49–64 of PDC-109 (CVFPFVYRNRKHFDCT), which is the FN2 domain (Greube et al, 2001). PDC-109 has been reported to stabilize the sperm membrane in a first step (Greube et al, 2001) and subsequently to participate in the female tract in capacitation by releasing cholesterol and binding

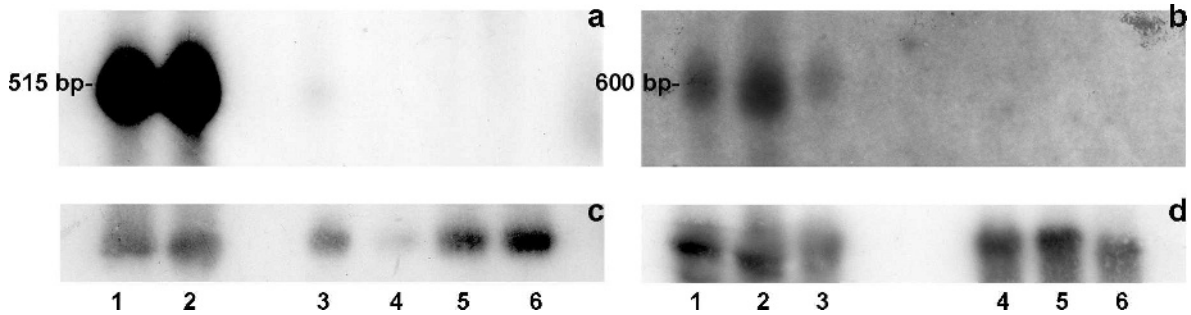

Figure 5. Expression of P14 **(a, c)** and P20 **(b, d)** detected by Northern blotting **(a, b)**, with the loading of RNA illustrated by the 18S rRNA on the membrane **(c, d)**. Extracted RNA was from different samples of seminal vesicles (**a:** lanes 1 and 2; **b:** lanes 1, 2, and 3) and vas deferens (**a:** lanes 3, 4, 5, and 6; **b:** lanes 4, 5, and 6).

high-density lipoprotein and heparin (Thérien et al, 1998, 2001; Gwathmey et al, 2003). However, the P20 fragment was not homologous with any reported protein (Barrios et al, 2005).

In the present study, we examined the localization and distribution of P14 and P20 in testis, prostate, seminal vesicles, vas deferens (the distal fraction including the ampullae), efferent ductules, bulbourethral glands, and 3 epididymal sections (caput, corpus, and cauda) of the ram. Western blot analyses showed that both proteins were detected only in the seminal vesicles and vas deferens. As these 2 parts are very close to each other, the signal detected in the vas deferens could be due to contamination with the SVS while the samples were taken. Results of immunohistochemical analysis by means of indirect immunofluorescence and avidin-biotin complex technique confirmed this hypothesis, as reactivity was exclusively found in seminal vesicles. Consistent findings were obtained by Northern blot analyses, as P14 and P20 were undetectable in vas deferens but present in seminal vesicles. These results demonstrate that the ram P14 and P20 genes are restrictedly expressed in the seminal vesicles, suggesting the important role of these genes in vesicular functions.

Furthermore, the immunohistochemical staining by the avidin-biotin complex technique showed that both proteins were expressed in the cytoplasm of the secretory epithelial cells, though not all the cells were in the same active state. The fact that some cells in contact with the lumen did not show reactivity in the cytoplasm suggests that they are resting cells from the secretory cycle. Indeed, transition forms were observed between highly active and inactive cell types.

The fusion of secretory membrane vesicles with the sperm plasma membrane may be one of the mechanisms involved in the transfer of some seminal plasma proteins to the sperm surface (Frenette and Sullivan, 2001). Using specific antibodies, Aumüller and Scheit (1987) identified the bovine seminal vesicles as the source of different secretory proteins of bull seminal plasma. One of these proteins, the PDC 109, was shown to specifically bind to the midpiece of epididymal spermatozoa that was interpreted as initiating the onset of sperm motility (Scheit et al, 1988).

Results of this investigation indicate that 2 seminal plasma proteins of approximately 14 (P14) and 20 (P20) kDa that protect ram spermatozoa against cold shock (Barrios et al, 2005) are secreted specifically in the seminal vesicles. On the basis of these results, we suggest referring to these 2 proteins as RSVP14 and RSVP20, respectively, according to their origin and molecular weight. Because of the high homology found between RSVP14 and bovine PDC 109 (both containing the FN2 domain) and the obtained evidence that P14 is partially released from the sperm membrane during capacitation and is redistributed over the sperm surface (Barrios et al, 2005), as reported for BSP proteins (Thérien et al, 2001), we have recently suggested that RSVP14 may take part in the protein structure surrounding the spermatozoa in a similar way as fibronectin, stabilizing membrane phospholipids and cytoskeleton (Barrios et al, 2005). We have found out by partition in a 2-phase system with Triton X-114 (Sigma) that these proteins are highly hydrophobic (data not shown), and it was confirmed by the obtained results of partial amino acid sequencing, especially P20 (Barrios et al, 2005). These hydrophobic domains might account for their fusion with the sperm surface. Furthermore, we have reported seasonal differences in the ability of ram seminal plasma proteins to recover membrane integrity of cold-shocked sperm, with an important decrease in several seminal plasma proteins of low molecular weight during the nonbreeding season (Pérez-Pé et al, 2001b).

These results should increase the understanding of the ram SVS composition and its putative role in the sperm functionality. Studies to define the role that RSVP14 and RSVP20 play in influencing ram fertility are currently under way.

Acknowledgments

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