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Epididymal α-L-fucosidase and its possible role in remodelling the surface of bull spermatozoa

Andrea Carolina Aguilera [a, b], Veronica Boschin [a], Inmaculada Robina [c], Pilar Elías-Rodríguez [c], Miguel Angel Sosa [a, b, *]

[a] Laboratorio de Biología y Fisiología Celular "Dr. Franciso Bertini", Instituto de Histología y Embriología – IHEM-CONICET-FCM-UNCuyo, 5500 Mendoza, Argentina
[b] Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina
[c] Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Spain

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ABSTRACT

The mammalian epididymis provides an appropriate environment for sperm maturation. During the epididymal transit, spermatozoa undergo biochemical and morphological changes that lead to the acquisition of the fertilizing capacity. In this study we analysed the fucosylation status of membrane glycoproteins in the spermatozoa obtained from different regions of the bull epididymis. High amounts of fucose were detected on caput spermatozoa (R.F.I. = 1010 ± 20.35), mostly located in the post-acrosome zone. A significant decrease in the fucose levels was detected toward the cauda (R.F.I. = 540.5 ± 49.93) ($P < 0.05$). This decrease was in line with the increased activity of α-L-fucosidase in the cauda fluid. In sperm from the cauda, the defucosylation occurred in some proteins, whereas others showed higher fucosylation rates. A significant decrease of fucose in the gametes was observed upon incubation of crude cauda fluid with caput spermatozoa (from R.F.I. = 1.45 ± 0.08 to 1.06 ± 0.03) ($P < 0.05$) indicating that the α-L-fucosidase present in the epididymal fluid is active on spermatozoa. Moreover, this effect was blocked with specific enzyme inhibitors. These results provide direct evidence that the α-L-fucosidase from epididymal fluid participates in the fucose removal from spermatozoa, as a step of sperm maturation in the bull epididymis.

1. Introduction

The mammalian epididymis plays an important role in the acquisition of motility and the fertilizing capacity of spermatozoa [1]. During the passage through the epididymis, spermatozoa undergo biochemical and morphological changes, resulting in a gamete that is competent for fertilization. These changes include adsorption of epididymal proteins on the sperm surface, a molecular reorganization of the plasmalemma and changes in the composition of glycoconjugates on the cell surface [2–7]. Thus, the maturation of spermatozoa in the epididymis is the result of sequential interactions with proteins secreted by the epithelium along the epididymal duct [8–13].

Among the several proteins secreted by the epididymal epithelium, acid glycosidases can be found at high concentrations in the epididymal fluid of different mammalian species [2,5,6,14–19]. Although the pH of the epididymal lumen is not optimum for acid hydrolase activity [20], it has been demonstrated that some enzymes, such as β-galactosidase is active in the fluid of the rat epididymis [21]. However, other functions for those glycosidases could be attributed from their interaction with specific receptors found in the plasmalemma of spermatozoa [7,22].

Glycosidases have also been found in the epididymis of domestic animals, such as bulls [23]. Among them, α-L-fucosidase (α-FUC) appears to be important for reproduction in bulls, since it was prevalent in the cauda epididymal fluid of highly fertile animals [24]. Moreover, dogs with severe deficiency of α-FUC have shown an impaired sperm maturation [25].

It has been proposed that α-FUC might contribute to modify the carbohydrate moieties of sperm membrane glycoproteins during the epididymal transit; however, this hypothesis remains to be confirmed. Mammalian α-L-fucosidases are a ubiquitous group of a large multimeric lysosomal glycosidases involved in the hydrolysis

* Corresponding author. IHEM-CONICET, Argentina.
E-mail address: msosa@fcm.uncu.edu.ar (M.A. Sosa).

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of α-L-fucose from a diverse group of fucoglycoconjugates [26]. Two isoforms of this enzyme have been described in bull reproductive tissues, although only the FUCA1 isoform has been found in the cauda epididymal fluid, the seminal plasma and spermatozoa [27]. Other studies have suggested that, in other mammalian species, the enzyme detected in the epididymal fluid and seminal plasma differs from the sperm membrane-associated enzyme [28–30].

Several studies support the involvement of fucose and/or fucosidases in the fertilization process, indicating that may play an important role in the association to the oviduct to form a sperm reservoir [31–33] or for gamete recognition [28,29,34,35]. In addition, the amount of fucose decrease after incubation with oviductal fluid [36]. The addition of this monosaccharide to an *in vitro* fertilization assay inhibits the penetration into bovine oocytes [37]. The α-L-fucosidases are involved in the biosynthesis of fucosylated glycoproteins and, therefore, inhibitors of this enzyme are important tools in the study of fucose-containing glycoconjugates that participate in fertilization processes [29].

Taking in account the importance of fucose after epididymal maturation, the aims of the present study were to determine whether the changes in the fucose content in the sperm surface during the epididymal transit can be taken as a maturation parameter, and if the secreted α-FUC is responsible of such modifications. Herein, we present direct evidences indicating that α-FUC from the cauda epididymal fluid may be responsible for the changes in the carbohydrate composition during sperm maturation in the epididymis.

2. Materials and methods

2.1. Reagents

The rabbit polyclonal antibody against α-L-fucosidase (FUCA 1) was purchased from Santa Cruz Biotechnology (sc-134716). The FITC-conjugated UEA-I (cat.L9006) and biotin-conjugated UEA-I (L8262) were purchased from Sigma-Aldrich (St. Louis, MO). The *p*-nitrophenyl-α-L-fucopyranoside substrate for α-fucosidase (Cat. No. 3628) was also purchased from Sigma-Aldrich (St Louis, MO). Nitrocellulose (NC) membranes (pore size 0.45 μm) were purchased from GE Healthcare (Germany). The chemiluminescent reagent was prepared with 1.25 mM luminol (Sigma-Aldrich, St Louis, MO), and 198 μM *p*-coumaric acid in 100 mM Tris-HCl (pH 8.5) according to Mruk and Cheng [38]. The α-L-fucosidase inhibitors, such as (2*S*,3*S*,4*R*,5*S*)-2-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-5-methylpyrrolidine-3,4-diol (C1), (2*S*,3*S*,4*R*,5*S*)-2-[1-((4-benzyloxycarbonyl-5-methylfuran-2-yl)-methyl)-1*H*-1,2,3-triazol-4-yl]-5-methylpyrrolidine-3,4-diol (C2) [39] and (2*S*,3*S*,4*R*,5*S*)-2-(1*H*-benzoimidazol-2-yl)-5-methylpyrrolidine-3,4-diol (C3) [40,41] were provided by Dr. Inmaculada Robina (University of Seville, Spain). All the inhibitors are five membered iminosugars (pyrrolidines) having an L-fuco configuration, and bearing an aromatic moiety attached at carbon 2 of the pyrrolidine backbone.

2.2. Animals

Reproductively mature bulls (*Aberdeen-Angus*, ~4 years old) were used in this study. The animal's fertility was certified by the supplying farms, where they had been used for reproductive purposes. Bulls were fed with diets primarily composed of pasture provided by farms located in the province of Córdoba (Argentina). All the procedures were performed according to the protocol approved by CICUAL (Committee for Animal Care of the Universidad Nacional de Cuyo, Mendoza, Argentina). These procedures also fulfilled the policies and regulations of the Argentine National Institute of Health and the National Research Council.

2.3. Biological samples

All procedures were carried out according to Aguilera et al. [7]. Briefly, after slaughtering, epididymides were removed and transported to the laboratory and processed immediately. Organs (one per animal) were carefully dissected and the caput, corpus and cauda were processed separately. To obtain epididymal tissue and spermatozoa, each organ (the three regions) was cut into small pieces with a stainless steel blade. Samples were suspended (1:3 w/v) in Hank's solution. Incubations were carried out, at 37 °C for 30 min with gentle manual agitation to allow for spermatozoa release. The minced tissue was settled for 10 min at 4 °C and the resulting supernatant (containing fluid and spermatozoa) was centrifuged at 400×*g* for 5 min and spermatozoa were separated from the fluid. The spermatozoa were washed three times with Hank's solution and finally pelleted at 1000×*g* for 10 min. The epididymal fluids were stored at −20 °C until used. The remaining epididymal tissue was homogenized with buffer H (10 mM Tris–acetate, pH 7.2, containing 0.25 M sucrose, 1% EDTA, 1 mM PMSF, 0.02% sodium azide, and 5 mM glycerophosphate) employing a Teflon/glass tissue homogenizer and centrifuged at 800×*g* for 20 min at 4 °C. The post-nuclear supernatant was stored at −20 °C until used.

2.4. Immunoblotting

All the procedures were carried out according to Aguilera et al. [19]. Briefly, 45 μg of proteins from the epididymal tissue or fluid were run on SDS–PAGE gels (10% acrylamide/bisacrilamide) and electrotransferred to nitrocellulose (NC) membranes at 250 V for 1 h. After transferring, the non-specific binding sites were blocked for 1 h with 6% skimmed milk in phosphate-buffered saline containing 0.05% Tween 20 (PBS-T). Membranes were then washed three times with PBS-T and incubated for 2 h with anti-FUCA 1 diluted 1:250 in PBS-T. Membranes were washed and incubated with the biotinylated anti-rabbit IgG antiserum (diluted 1:5000 in PBS-T) for 2 h. After washing as above, NC membranes were incubated with peroxidase-conjugated streptavidin (1:10000 in PBS-T) for 1 h and washed again. Specific bands were detected by the enhanced chemiluminescence method, and quantified by densitometric scanning of membranes using an Image Quant LAS 4000 scanner (GE Healthcare, Uppsala, Sweden). NC membranes were stained with Ponceau S as loading and transference controls.

2.5. Detection of fucose on nitrocellulose immobilized glycoproteins

Proteins from caput or cauda sperm membrane were extracted with a hypoosmotic solution according to Jeyendran et al. [42]. Briefly, spermatozoa (up to 1×10^7spermatozoa/ml) were mixed with a hypoosmotic solution (25 mM sodium citrate dihydrate and 75 mM fructose) and incubated for 2 h at 37 °C. Gametes were sonicated over ice until spermatozoa tails were separated from the rest of the cell and centrifuged at 4000 g for 15 min. Pellets, corresponding to intact spermatozoa, were discarded and the supernatants (SN1) were centrifuged at 10000×g for 30 min. Pellets (enriched-membrane fractions) were resuspended in 0.05 M Tris-HCl buffer (pH 7.2), containing 0.5% saponin, 50 mM EDTA and 1 mM PMSF. Proteins (45 μg) were run on 10% SDS-PAGE gels under non-reducing conditions and electrotransferred to NC membranes for 90 min at 250 V. After transferring, non-specific binding sites were blocked for 1 h with 6% skimmed milk in PBS-T. Membranes were then washed three times with PBS-T and incubated for 2 h with the biotin-conjugated lectin UEA-I (5 μg/ml in PBS-T). Membranes were washed as described above and incubated with peroxidase-conjugated streptavidin (1:10000 in PBS-T) for 1 h.

After washing with PBS-T, the lectin-reactive bands were detected with the enhanced chemiluminescence method and quantified by densitometric scanning on the membranes using an Image Quant LAS 4000 scanner.

2.6. Fluorescence microscopy

To evaluate the fucose amount on the sperm surface, the spermatozoa obtained from caput and cauda (as described above) were resuspended in PBS/PVP (0.1% polyvinylpyrrolidone in PBS) and fixed with 2% *p*-formaldehyde (PAF) in PBS for 10 min. Cells were then placed on slides (previously treated with 10 mM poly-lysine for 30 min) to perform the immunofluorescence method. To this end, slides containing spermatozoa were blocked for 1 h at room temperature with 5% horse serum in PBS/PVP and then incubated with 100 μl UEA-I lectin (10 μg/ml) in PBS/PVP containing 1% horse serum. After three washes with PBS/PVP, slides were mounted on glass coverslip with UltraCruz™ mounting medium (USA). Slides were then examined with in a Nikon 80 fluorescence microscope (Japan).

2.7. Flow cytometry

Gametes from caput and cauda were washed three times with Hank's buffer and centrifuged at 1000×*g* for 10 min. Samples were fixed with 2% PAF in PBS/PVP for 10 min at room temperature and then blocked for 1 h at 37 °C with 5% horse serum (in PBS/PVP) and washed three times with PBS/PVP. Each sample (5×10^5 cells) was incubated with FITC-conjugated UEA-I (5 μg/ml) in 100 μl final volume for 2 h at room temperature in the dark. The specificity of lectin binding was controlled by incubation in the presence of 0.5 M fucose. The sugar concentration was determined in previous experiments. Gametes were then washed twice with PBS/PVP, and the number of the lectin reactive sperm was analysed on a BD FACSAria III (USA) flow cytometer and the FACS Diva Software. In all cases, a gate was established using dots plots of forward scatter (FSC) *vs.* side scatter (SSC) plots, since the same cellular and physical properties (size and granularity) was obtained for all treatments. After excitation at 488 nm, the emission was detected employing a 525 nm band pass filter. The percentage of the spermatozoa that reacted with FITC-conjugated lectins and the mean fluorescence intensity was analysed using the FlowJo software version 7.6.2.

2.8. Incubation of caput sperm with cauda fluid

Caput spermatozoa were obtained as described above and the crude cauda epididymal fluid was obtained by gentle retrograde perfusion with Hank's buffer from the deferent duct until a drop with the luminal content was observed. Four drops of each epididymis were collected in Eppendorf tubes. After centrifugation at 2000 rpm, supernatants were recovered and used in the assay. For the assay, gametes were previously washed three times with Hank's buffer at 33 °C. Spermatozoa (5×10^5 cells) were then mixed with 100 μl of the crude epididymal fluid and incubated for 3 h at 33 °C. To confirm the enzyme specificity, the assays were carried out in the presence of the following FUCA 1 inhibitors at the final concentrations of 0.75 mM of the benzyltriazole pyrrolidine (C1), 0.1 mM of the benzyloxicarbonyl triazole pyrrolidine (C2), 0.43 mM of the imidazolil pyrrolidine (C3). These conditions had previously been determined in previous works according to the IC_{50} values [39–41]. As controls, gametes were incubated with either Hank's buffer alone or with inactivated fluid (15 min at 70 °C). After incubation, gametes were washed three times with Hank's buffer and prepared for subsequent lectin binding and flow cytometry.

2.9. Enzymatic assays

The activity of α-Fuc was measured using *p*-nitrophenyl -α-L-fucopyranoside substrate according to Barrett and Heath [43]. One unit of enzymatic activity catalyzes the release of 1 nmol of *p*-nitrophenol. Different buffer solutions were used depending on the experiment: 0.2 M citrate buffer was used to determine the activity at low pH values (4.5, 5 and 5.5) and 0.2 M phosphate buffer was employed to the determine the activity at high pH values (6, 6.5 and 7). The protein concentration was determined according to Lowry [44].

2.10. Statistical analyses

Data were analysed for normality distribution before a *t*-test or one-way ANOVA was performed. A Dunnett's multiple comparisons test or a Tukey-Kramer's multiple comparisons test was used after one-way ANOVA as indicated in each experiment. The level of significance was set at $p < 0.05$.

2.11. Experimental design

Experiment 1. Qualitative and quantitative evaluation of fucose in caput and cauda epididymal spermatozoa. For fucose content, FITC labelled lectin UEA-I followed by fluorescence microscopy and flow cytometry was used. In addition, we analysed the pattern of fucosylated proteins by incubating biotin labelled lectin UEA-1 with sperm proteins immobilized in Nitrocellulose membrane.

Experiment 2. Evaluation of α-FUC content in the epididymis. The expression of α-FUC was studied by western blot on proteins from the tissue and fluid of the caput, corpus and cauda epididymis. Moreover, we tested the activity of this enzyme by spectrophotometrically, using a specific colorimetric substrate (*p*-nitrophenyl -α-L-fucopyranoside).

Experiment 3. Effect of the cauda epididymal fluid on fucose content of caput spermatozoa. To examine the ability of the cauda fluid to remove fucose from the caput spermatozoa, we incubated a crude cauda fluid with the spermatozoa under conditions of epididymal environment. Afterwards, we evaluated the fucose content by flow cytometry. As control, a heat inactivated (70° C) cauda fluid was used.

Experiment 4. Effect of α-FUC inhibitors on the fucose content of caput spermatozoa. To corroborate the involvement of the soluble cauda epididymal α-Fuc in the fucose removal of the caput spermatozoa, we incubated the samples in the presence of specific inhibitors for the enzyme. The fucose content was then evaluated by flow cytometry.

3. Results

In this study, we analysed the presence and location of α-L-fucose in sperm plasma membrane in bulls during their epididymal transit. By using FITC labelled lectin UEA-I, we observed a post-acrosomal fucose detection in the caput spermatozoa, which becomes lower (or weaker) towards the cauda gametes (Fig. 1A). These results were quantified by flow cytometry and we observed a significant decrease of fucose content in the surface of epididymal spermatozoa from the caput to cauda (Fig. 1 B). The specificity of the lectin was confirmed by competitive inhibition with the sugar L-fucose (Fig. 1B). In addition, the pattern of fucosylated proteins was evaluated by binding of biotinylated UEA-I to sperm proteins immobilized on nitrocellulose membranes (see materials and methods). In agreement with the previous results, a decrease in the fucosylation level was observed in most of the proteins of cauda spermatozoa (in the range of 75–110 and 30-20 kDa) when

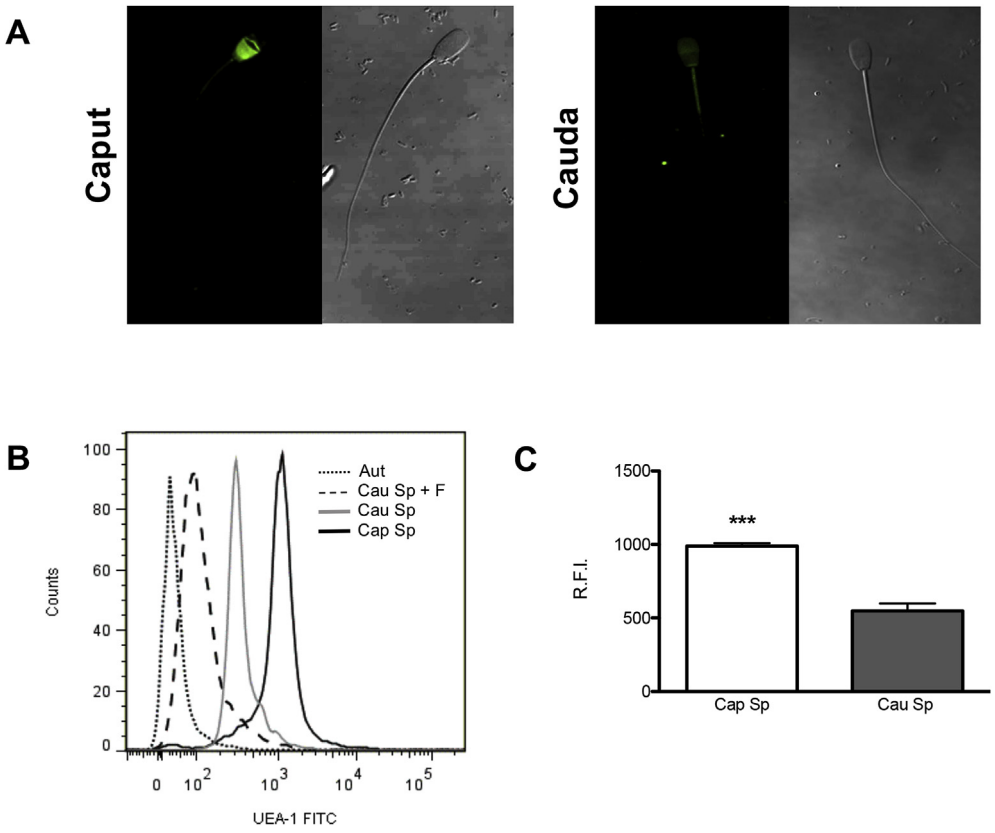

Fig. 1. α-L-fucose in bull epididymal spermatozoa. A) Distribution of the sugar in spermatozoa from caput and cauda using FITC-conjugated UEA-I and assessed by fluorescence microscopy. B) Analysis of the fluorescence by flow cytometry, and C) the corresponding quantification. Aut: autofluorescence of cauda spermatozoa alone; Cau Sp: cauda spermatozoa; Cau Sp + F: cauda spermatozoa plus 0.5 M fucose; Cap Sp: caput spermatozoa. Bars represent the means of relative fluorescence intensity (R.F.I.) ± SD (n = 12). Data were analysed by *t*-test, (***) significantly different from cauda ($P < 0.05$).

compared with the caput spematozoa, although an increase in other protein of lower Mr (~50 kDa) was observed in cauda spermatozoa (Fig. 2). The decreased fucosylation in the cauda spermatozoa may be related to an increase of α-FUC activity along the epidiymal duct. Therefore, we evaluated the presence and the activity of α-FUC in the bull epididymis to find that the enzyme is mostly expressed in the caput and cauda and also in the corpus though at a lesser extent (Fig. 3 A). Interestingly, the enzyme was mostly detectable in the fluid obtained from the cauda epididymis (Fig. 3 B). These results were in agreement with the measured enzymatic activity in each epididymal region (Fig. 3C). For these reasons, we decided to test whether this enzyme can be active on caput spermatozoa and the responsible of fucose remotion under the conditions of epididymal environment. First, we observed that epididymal α-FUC is active against synthetic substrates at the epididymal pH (6.5) although only 50% of the optimum (pH 4.5) (data not shown). Then we developed an assay in which the crude cauda fluid was incubated with caput spermatozoa at 33 °C.

After incubation, the fucose content was evaluated by flow cytometry, and we observed a significant decrease in the sugar amount (Fig. 4), indicating that the enzyme can remove fucose from the caput spermatozoa. This effect was not observed when heat inactivated epididymal fluid (70 °C) was used (Fig. 4). To corroborate that α-FUC activity from the epididymal fluid was responsible for the decrease in the fucose content, the cauda spermatozoa were incubated with the caput fluid in the presence of specific inhibitors, such as C1, C2 and C3 (Fig. 5 A). As shown in Fig. 5 B, the three compounds were able to inhibit the sperm defucosylation caused by the epididymal fluid, thus confirming that an α-FUC is involved

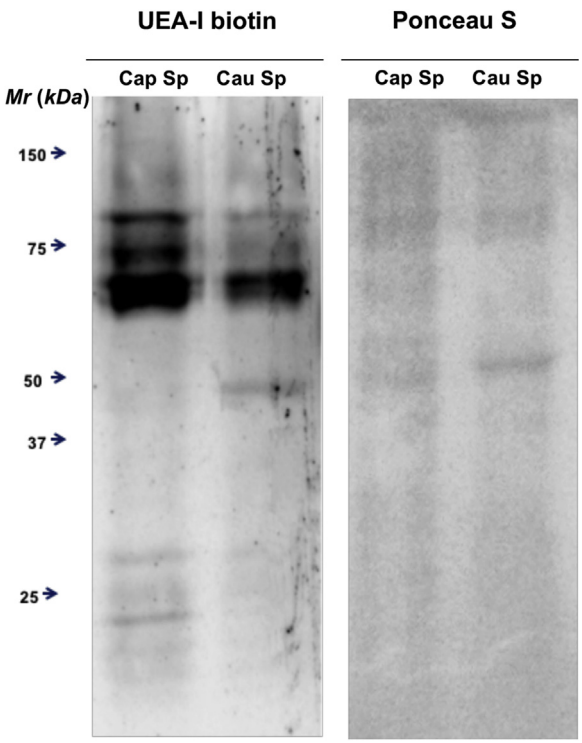

Fig. 2. Patterns of membrane fucosylated proteins from caput (Cap Sp) and cauda (Cau Sp) spermatozoa, detected with biotin-conjugated UEA-I on nitrocellulose membranes. As control, the membrane was stained with Ponceau S.

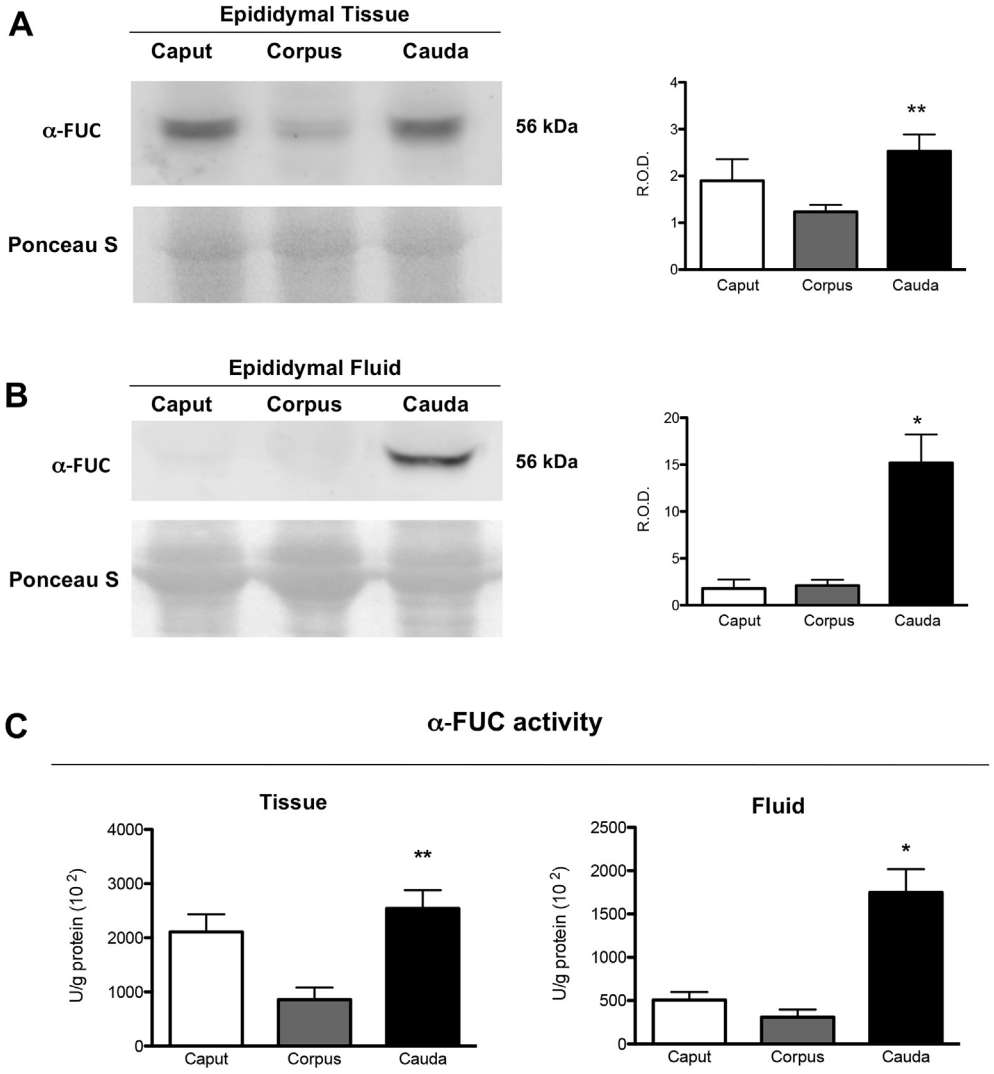

Fig. 3. Detection of α-L-fucosidase (α-FUC) in bull epididiymis. Immunoblot for detection of α-FUC in epididymal tissue (A) and fluid (B), and the corresponding quantifications from the epididymal regions as indicated. Bars represent the means of relative optical densities (R.O.D.) ± SD (n = 3). Ponceau S stain was used as loading and transference controls. (*) Significantly different from corpus and caput ($P < 0.05$), and (**) significantly different from corpus ($P < 0.05$C). α-FUC activity measured in epididymal tissue and fluid from the three epididymal regions (C). Bars represent the means ± SEM of specific activity (n = 5). (*) Significantly different from corpus and caput ($P < 0.05$) and (**) significantly different from corpus ($P < 0.05$). In all the cases, data were analysed by one-way ANOVA followed by a Tuckey-Krameŕs multiple comparison test.

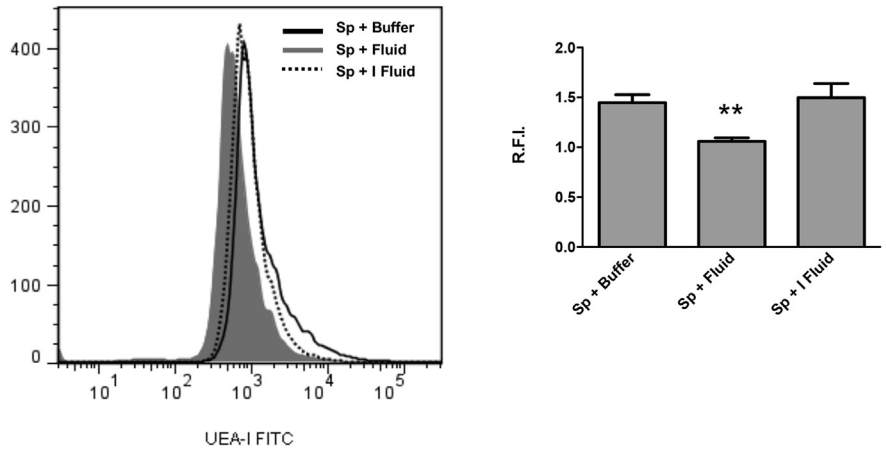

Fig. 4. Determination of α-L-fucose in caput spermatozoa after the incubation with cauda fluid. Flow cytometry analysis of the fucose content using the FITC labelled lectin UEA-I after the incubation of caput spermatozoa (Sp) with cauda fluid. As control, cauda inactivated fluid (I Fluid) (heated for 15 min at 70 °C) and buffer (Hank's) were used. Bars represent the means of the R.F.I ± SEM (n = 12) of each treatment. Data were analysed by one-way ANOVA followed by a Dunnett's multiple comparison test. (**) Significantly different from Buffer and I Fluid ($P < 0.05$).

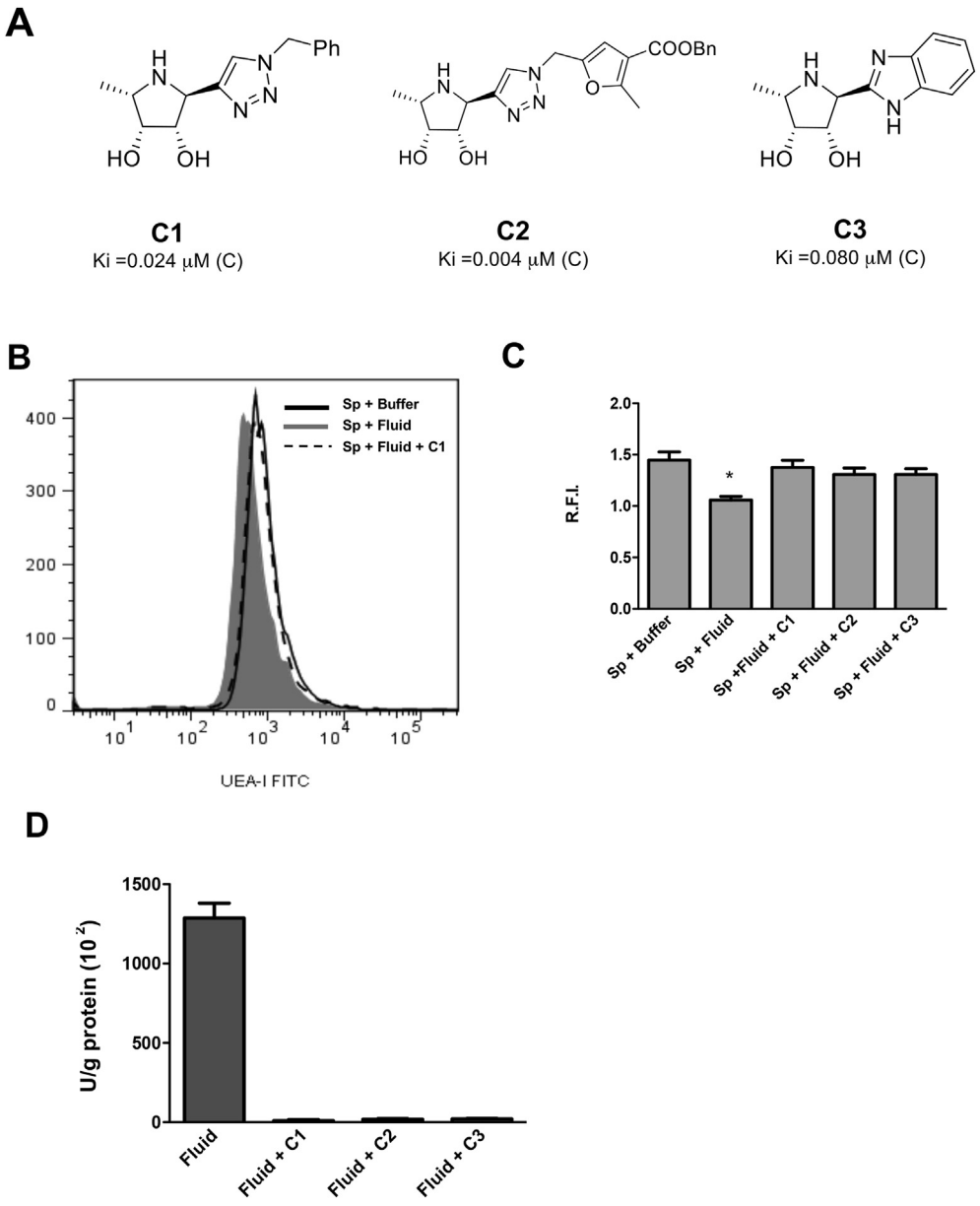

Fig. 5. Determination of α-L-fucose in caput spermatozoa (Sp) after the incubation with cauda fluid, in the absence or presence of the α-FUC inhibitors. A) Molecular structure of the synthetic inhibitors (C1, C2 and C3) of α-L-fucosidase (α-FUC). Ki represents the inhibition constant for each compound. B) Flow cytometry analysis, the profiles are representative of all the conditions employed and C) quantification of R.F.I. Data were normalized to fluid alone (R.F.I. = 1). Bars represent the means of RFI ± SD (n = 12). Data were analysed by one-way ANOVA followed by a Dunnett's multiple comparison test. (*) Significantly different from all the conditions used ($P < 0.05$). D) The effect of the inhibitors (at 0.3 μM, 17 nM and 80 nM for C1, C2 and C3, respectively) on the α-FUC activity of cauda epididymal fluid (n = 5). Bars represent the means ± SEM of specific activity.

in this phenomenon. In addition, the specificity of the inhibitors was confirmed by evaluating enzyme activity with a synthetic substrate (Fig. 5C).

4. Discussion

The mammalian epididymis provides the proper molecular environment for the sperm maturation. During this process, the plasma membrane undergoes several modifications that include changes in the carbohydrate composition. In this study, we present evidences indicating that the fucose content on the sperm surface is modified during the epididymal transit and that this phenomenon could be related to sperm maturation in bulls.

Herein, we observed that most of the fucosylated proteins are located along the post-acrosomal region in the caput spermatozoa. Despite the total fucose content on sperm membrane decreases from the caput to the cauda, some proteins increase their fucosylation status. This phenomenon could be the result of the balance between the activities of α-L-fucosidase (α-FUC) and fucosyltransferase existing in the epididymal fluid. The presence of glycosyl-transferases has been documented in the mammalian epididymis [2,6,45]. Moreover, it is known that the mammalian epididymis cells synthesize and secrete acid glycosidases into the lumen, which could be responsible for the changes observed in the sperm surface [2,5,6,14–19]. However, there were few direct evidences that demonstrate a role of the enzymes in this process. In previous studies, we have described the presence of some of these enzymes in the epididymal lumen and also observed that some of

them interact with specific receptors on the sperm plasma membrane [7]. However, α-L-fucosidase mostly found in the soluble form, but not associated to spermatozoa neither within membrane bounded vesicles from epididymal fluid, likely epididymosomes (unpublished data).

Curiously, the pH of the epididymal environment would not be favourable for the enzymatic activity, since the glycosidases are lysosomal enzymes. In this work, we have focussed our study on the role of α-FUC in the remodelling of the sperm surface and have provided direct evidences indicating that the α-FUC present in the cauda epididymal fluid is active at conditions similar to those of the epididymal environment, being able to remove fucose from the surface of bull spermatozoa. In previous studies, the α-FUC activity was associated with the reproductive capacity, since this enzyme increases in the epididymis of reproductively mature animals with respect to immature ones, and it is mostly redistributed to the epididymal fluid in the cauda region (Aguilera et al., unpublished data). These evidences allow suggesting a role of this enzyme in the fertility development in bulls. Furthermore, these results were in accordance with those of Moura et al. [24], who have demonstrated the existence of a high content of α-FUC in the cauda fluid of bulls with high fertility capacity. By means of a polyclonal antibody against α-FUC, we detected a single band of 56 kDa, suggesting the existence of one isoform in the epididymal fluid as in other species [29,30]. However, the presence of other isoforms cannot be ruled out, since α-FUC associated to sperm membrane has been described in bulls and in other mammalian species [7,28–30,46,47]. The fact that the expression and activity of α-FUC are detected in the cauda epididymis may indicate that most of the defucosylation process occurs in this region of the organ. The epididymal α-FUC also exhibits some properties that are similar to those from other tissues, such as an optimal acidic pH and sensitivity to synthetic specific inhibitors [29,39,40]. However, the epididymal α-FUC is also active, although at a lesser extent, at a pH that is similar to the epididymal environment. Taking into account this phenomenon, we performed an *in vitro* assay in which we incubated spermatozoa from the caput with the crude fluid from the cauda. Under these experimental conditions, we clearly demonstrated that α-FUC from the fluid is able to remove fucose from the sperm surface. The involvement of the enzyme in this process has been corroborated by the use of specific inhibitors which are active against the epididymal enzyme. It has been suggested that fucose may play an important role in fertilization, since the addition of this monosaccharide or fucoidan to the fertilization medium causes a reduction in the penetration rates to porcine and bovine oocytes [37,48]. Other studies suggest that fucose is involved in the formation of the sperm reservoir in the oviduct [31–33]. It is also likely that sperm surface undergo additional changes in carbohydrate composition once they leave the epididymis. This could be the result of the enzymatic activity of glycosidases that have been described in reproductive fluids, such as oviductal fluid [49].

These data provide, for the first time in bulls, direct evidence that the soluble α-FUC in the epididymal fluid participates in the remodelling of the sperm surface, as a step in the maturation of gametes, and that the fucosylation status of sperm proteins contributes, in part, to that maturation process. Thus, the activity of α-FUC and the state of fucosylation of sperm proteins can be considered as a parameter of maturation. The latter finding might be useful in future studies assessing dysfunctions related to male infertility.

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