

Vicuna oviduct mucosa: Ultrastructure and lectin affinity

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Abstract

The isthmus and uterotubal junction (UTJ) from oviducts of adult female vicunas (*Vicugna vicuna*) were used to study the epithelial cell ultrastructure. Scanning microphotographs revealed a mosaic pattern made up of ciliated and secretory cells with abundant microvilli. In the isthmus only a few secretory cells were present, scattered among numerous ciliated cells while in the UTJ the secretory cells prevail. The abundance of secretory cells suggests that the UTJ cells may possess special characteristics for sperm binding. No studies exist in either the vicuna or any of the other South American Camelids (SAC) concerning carbohydrates involved in sperm or embryo interactions on the oviductal surface. Identification of these sugars seems relevant to the mechanism involved in the sperm–oviduct interaction. As a first step in the determination of the molecular mechanism implicated in the formation of sperm reservoirs in the oviduct, lectin affinity for the oviductal mucosa was studied. High concentrations of glycosaminoglycans inside the two types of epithelial cells, and on their glycocalyx were observed with histochemical methods. The carbohydrates on the epithelial surface were labeled with fluorescent by lectins and analyzed with confocal scanning microscopy. The cell surface showed abundant α -mannopyranosyl, α -glucopyranosyl, β -galactosyl, *N*-acetyl glucosamine, and *N*-acetylneuraminic acid residues and few α -linked *N*-acetyl galactosamine residues. Neither α -L-fucopyranosyl nor β -*N*-acetyl galactosamine residues were observed in any part of the oviduct. The distinct ultrastructural characteristic of the UTJ as well as the presence of high concentrations of sugar residues on the mucosa surface of this portion of the oviduct could be related to its function as a sperm reservoir.

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1. Introduction

The caudal isthmus and uterotubal junction (UTJ) have been shown to be the oviductal reservoir for sperm in pigs, sheep and cattle (Hunter, 1984; Hunter and Nichl, 1983). The reservoirs ensure the fertility of sperm until

ovulation and are particularly relevant in reflex ovulator animals such as the South American Camelids (SAC). It seems that sperm binding to the oviductal epithelium involves carbohydrate recognition by means of an interaction between these glycoconjugates and the surface-associated sperm lectins (Töpfer-Petersen et al., 2002). This interaction would occur in a species-specific manner (Demott et al., 1995; Gwathmey et al., 2003).

As no studies exist concerning oviductal surface carbohydrates involved in the formation of a sperm reservoir

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in the oviduct of the SAC, the aim of this work was to describe the ultrastructure and lectin affinity of the oviducal mucosa of adult female vicunas (*Vicugna vicugna*), focussing on the isthmus and UTJ.

2. Materials and methods

2.1. Collection of oviducts

Oviducts were obtained from two sexually mature females vicuna (*V. vicugna*) killed accidentally during the gathering for shearing in INTA NOA Abra Pampa, Jujuy Province, Argentina. Although both oviducts were dissected, only the oviduct ipsilateral to the ovary with dominant follicles (6–7 mm) was used. The UTJ and the isthmus were dissected into 0.5 cm segments and fixed for later processing.

2.2. Scanning electron microscopy (SEM) of the oviductal mucosa

The oviductal segments were opened to expose the mucosal surface, fixed with Karnovsky solution and then processed at the Electron Microscopy Laboratory (LAMENOA) for observation via a JEOL CF 35 scanning electron microscope (Ball, 1996).

2.3. Histochemical detection of carbohydrates in the oviduct segments

The isthmus and the UTJ were fixed with paraformaldehyde buffered at 4% and then embedded in paraplast. The 7 μm sections were stained with periodic acid-Schiff reagent (PAS) (Suarez et al., 1997). Another group of slides was stained with AB/PAS using a 1% Alcian Blue (AB) solution 8Gx (Biopack), pH 2.5, for 25 min, before PAS staining. Counter-staining was effected with Harris' hematoxylin. Slides were evaluated using an Olympus Bx40 microscope.

2.4. Lectin labeling of oviductal tissue sections

Triticum vulgaris/wheat germ agglutinin (WGA), *Ulex europaeus* agglutinin 1 (UEA), *Dolichos biflorus* agglutinin (DBA), *Ricinus communis* agglutinin 120 (RCA), *Canavalia ensiformis* agglutinin (Con A), *Phaseolus vulgaris* agglutinin (PHA E and PHA L) and *Sophora japonica* agglutinin (SJA), conjugated with fluorescein isothiocyanate (FITC), FITC rhodamine or tetramethylrhodamine isothiocyanate (TRITC) were used for lectin labeling. The following pairs of labels were made: Con A—DBA; RCA 120—PHA E; UEA 1—SJA and WGA—PHA L. Confocal laser scanning

microscopy (CLSM), applying a Zeiss LSM 510, was used for examination.

The tissue sections were deparaffinized with xylol/isopropanol and rehydrated in graded ethanol dilutions. The sections were then washed several times with 0.01 M Tris-buffer, pH 7.4, before treatment with the first lectin. The sections were covered with each lectin solution (12 $\mu\text{g}/\text{ml}$ 0.01 M Tris-buffer) and placed in a wet chamber at room temperature in the dark for 60 min. Prior to addition of the second lectin, the sections were washed again with 0.01 M Tris-buffer. Nuclei were stained with 4',6-diamidine-2'-phenylindole dihydrochloride 1:1000 in PBS for 5 min in the dark and then they were washed in Tris-buffer and distilled water and mounted with Gel/Mount (Biomeda).

3. Results

Longitudinal folds and a system of lower secondary folds, ridges and chords projected into the central located

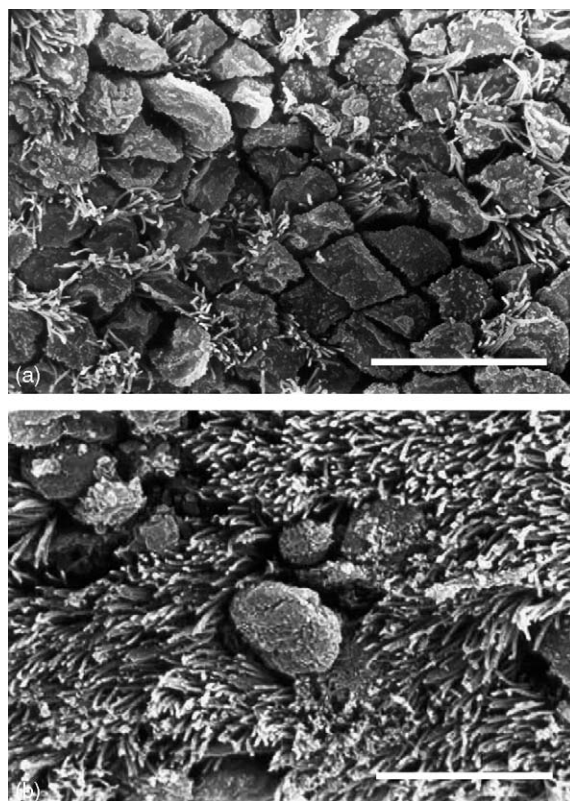


Fig. 1. Mucosa surface characteristics of the uterotubal junction (UTJ) and isthmus by scanning electron microscopy. Note the alternation between ciliated and secretory cells. Ciliated cells exhibit abundant cilia and secretory cells numerous microvilli. As regards the relative amount of either cell type. (a) In the UTJ a larger number of secretory cells is observed. (b) In the isthmus ciliated cells prevail. Bar: 10 μm .

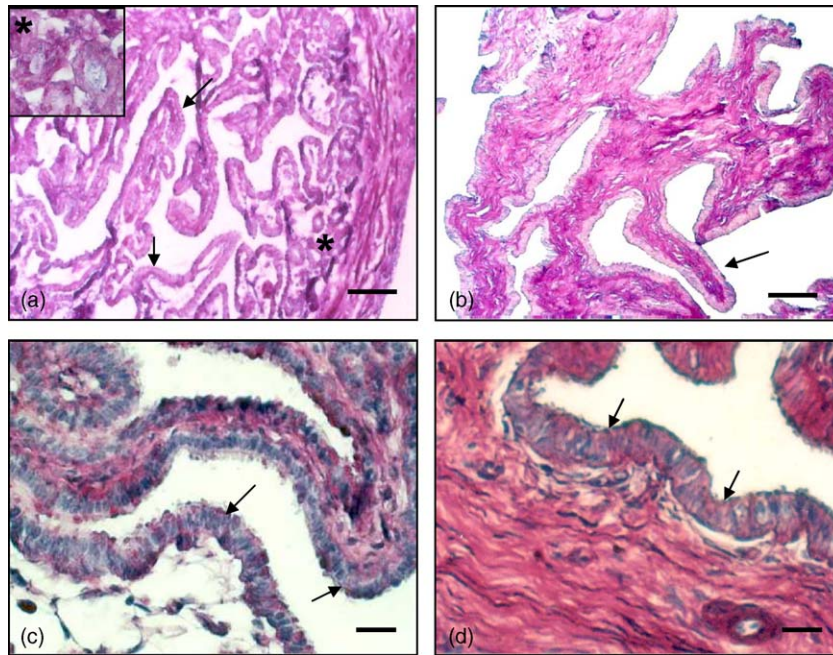


Fig. 2. Section of the uterotubal junction (UTJ) and isthmus of vicuna (a) PAS/AB/hematoxylin-stained isthmus; bar: 100 μ m. (b) PAS/AB/hematoxylin-stained UTJ. Bar: 100 μ m. (c) PAS/hematoxylin-stained isthmus; bar: 20 μ m. (d) PAS/hematoxylin-stained UTJ; bar: 20 μ m. Arrows indicate the apical border of the epithelium.

lumen were observed throughout the vicuna oviduct. The isthmus showed a complex branching pattern formed by the longitudinal folds of the mucosa. The UTJ is a transition area between the oviduct and the uterine horn, which ends on a small papilla that projects into the uterus. The UTJ is characterized by a submucosal layer, which is heavily vascularized and contains the first uterine glands. The musculature of the vicuna UTJ is characterized by a continuous circular or spiral intermediate layer, flanked by broad longitudinal inner and thin longitudinal outer layers. Scanning electron microscopy

of the luminal surface of the epithelial cells in the isthmus and UTJ during growth phases throughout the follicular wave of the ovarian cycle revealed two distinctive cells: ciliated and secretory cells (Fig. 1). The prevailing cells in the isthmus epithelium were densely ciliated while those in the UTJ belonged to the secretory type showing many microvilli on their bulbous processes.

PAS and AB staining revealed glycosaminoglycans on the oviductal mucosa (Fig. 2). AB staining showed a strong reaction of acid glycosaminoglycans throughout the glycocalyx of oviductal cells, which was par-

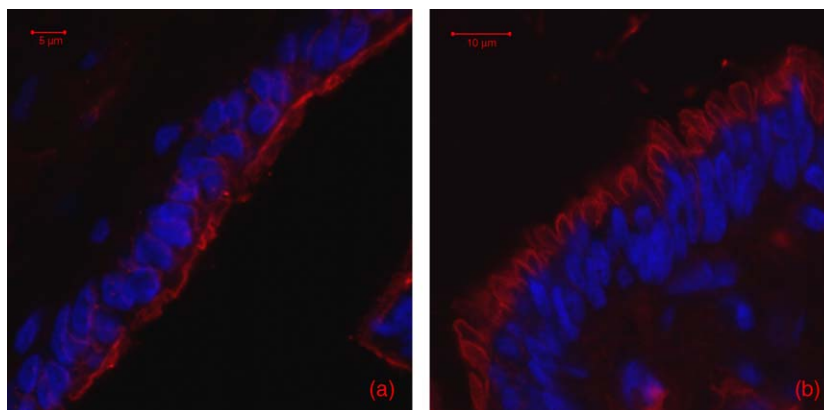


Fig. 3. Rhodamine labeled lectin (RCA 120) affinity of isthmus and uterotubal junction (UTJ) mucosa. (a) Isthmus; bar: 5 μ m and (b) UTJ; bar: 10 μ m.

ticularly intense in the basal region of the secondary folds. Fluorescent lectins used to determine the different sugars present in the glycocalyx of cells from the different oviductal segments failed to detect differences between the isthmus and UTJ (Fig. 3). Five of the eight FITC or rhodamine labeled lectins tested, demonstrated binding patterns along the oviduct. WGA and Con A reacted strongly with the glycocalyx of vicuna oviductal epithelial cells in a selective way. The binding patterns point to the presence of abundant α -mannopyranosyl, α -glucopyranosyl, β -galactosyl, *N*-acetyl glucosamine and *N*-acetylneuraminic acid residues. α -*N*-Acetyl galactosamine residues were sparse and no α -L-fucopyranosyl or β -*N*-acetyl galactosamine residues were detected.

4. Discussion

The ultrastructural morphology of the oviduct is closely related to its function. In the present study, the ultrastructure and cytochemical properties were examined using different techniques. As in other mammals (Hunter et al., 1987, 1991; Ball, 1996), the vicuna oviductal epithelium consists mainly of ciliated and secretory cells. Staining of the mucosa with AB at a pH 2.5 and with PAS indicated the presence of mucopolysaccharides throughout the isthmus and UTJ during the follicular growing phases. Both PAS and AB react to more than one molecular species, so the material stained could be composed of different mucopolysaccharides. Apparently, the oviduct mucus may provide a sticky surface for sperm storage and the intensity of staining provides an estimate of the viscosity of the fluid or density of mucopolysaccharides encountered by the sperm in the oviduct. Several mechanisms have been proposed to account for the storage of sperm in the UTJ and/or caudal isthmus (Harper, 1994). These include inhibition of sperm motility and obstruction of sperm ascent by mucus binding of sperm to mucosal epithelium. The latter theory has recently received more attention. However, the PAS/AB cells staining did not indicate a regional difference in the class of mucopolysaccharides present on the luminal surface and lectins affinity to the surface glycocalyx did not show a distinct pattern in the different segments of the oviduct. Nevertheless, It should be considered that our study only included two animals, whose follicular wave was in the growth phase as judged from their ovary follicular characteristics (Chaves et al., 2000). The expression of certain glycoconjugates in the oviduct can vary during sexual cycle as was demonstrated in horses (Desantis et al., 2004). Other studies at different hormonal stages are required in order to demonstrate that modifications in the gly-

coconjugates could be linked to different physiological conditions.

The interaction between sperm and oviductal cells seems to occur in a species-specific manner. In hamsters, it is mediated by sialic acid (Demott et al., 1995) and in horses by galactose (Lefebvre et al., 1995), while bovine sperm binds to fucosylated ligands (Gwathmey et al., 2003). In pigs' maltose, lactose and mannose are involved (Green et al., 2001). According to our results, the vicuna oviductal mucosa does not show α -L-fucopyranosyl or β -*N*-acetyl galactosamine residues, which probably indicates that these sugars are not involved in the vicuna sperm–oviduct interaction.

5. Conclusion

The present study represents the first report on the ultrastructure and glycocalyx composition of a vicuna oviduct. The secretory and ciliated cell differential pattern of UTJ indicates that it could play a special role during sperm or embryo transit through the duct. However, analysis of mucopolysaccharides and lectin affinity of the isthmus and UTJ luminal surface did not indicate functional differences of this segment.

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References

- Ball, B.A., 1996. Scanning electron microscopy of the equine oviduct and observations on ciliary currents in vitro at day 2 after ovulation. *Theriogenology* 46, 1305–1311.
- Chaves, M.G., Aba, M., Agüero, A., Egey, J., Berestin, V., Rutter, B., 2000. Ovarian follicular wave pattern and the effect of exogenous progesterone on follicular activity in non-mated llamas. *Anim. Reprod. Sci.* 69, 37–46.
- Demott, R.P., Lefebvre, R., Suarez, S.S., 1995. Carbohydrates mediate the adherence of hamster sperm to oviductal epithelium. *Biol. Reprod.* 52, 1395–1403.
- Desantis, S., Acone, F., Corriero, A., Deflorio, M., Zubani, D., Ventriglia, G., Palmieri, G., De Metrio, G., 2004. Distribution of sialoglycoconjugates in the oviductal isthmus of the horse during anoestrus and pregnancy: a lectin histochemistry study. *Eur. J. Histochem.* 48 (4), 403–412.
- Green, C.E., Bredl, J., Holt, W.V., Watson, P.F., Fazeli, A., 2001. Carbohydrate mediation of boar sperm binding to oviductal epithelial cells in vitro. *Reproduction* 122, 305–315.
- Gwathmey, T.M., Ignatz, G.G., Suarez, S.S., 2003. PDC-109 (BSP-A1/A2) promotes bull sperm binding to oviductal epithelium in vitro and may be involved in forming the oviductal sperm reservoir. *Biol. Reprod.* 69 (September (3)), 809–815.

- Harper, M.J.K., 1994. Gamete and zygote transport. In: Knobil, E., Neill, J.D. (Eds.), *The Physiology of Reproduction*. Raven Press Ltd., New York, pp. 123–187.
- Hunter, R.H.F., Nichl, R., 1983. Transport of spermatozoa in the sheep oviduct preovulatory sequestering of cells in the caudal isthmus. *J. Exp. Zool.* 228, 121–128.
- Hunter, R.H.E., 1984. Preovulatory arrest and periovulatory redistribution of competent spermatozoa in the isthmus of the pig oviduct. *J. Reprod. Fertil.* 72, 203–211.
- Hunter, R.H.F., Fléchon, B., Fléchon, J.E., 1987. Pre- and periovulatory distribution of viable spermatozoa in the pig oviduct: a scanning electron microscope study. *Tissue Cell* 19 (3), 423–436.
- Hunter, R.H.F., Fléchon, B., Fléchon, J.E., 1991. Distribution, morphology and epithelial interactions of bovine spermatozoa in the oviduct before and after ovulation: a scanning electron microscope study. *Tissue Cell* 23, 641–656.
- Lefebvre, R., Demott, R.P., Suarez, S.S., Samper, J.C., 1995. Specific inhibition of equine sperm binding to oviductal epithelium. *Biol. Reprod.* 1, 689–696.
- Suarez, S., Brockman, K., Lefebvre, R., 1997. Distribution of mucus and sperm in bovine oviducts after artificial insemination: the physical environment of the oviductal sperm reservoir. *Biol. Reprod.* 56, 447–453.
- Töpfer-Petersen, E., Wagner, A., Friedrich, J., Petrunkina, A., Ekhlesi-Hundrieser, M., Waberski, D., Drommer, W., 2002. Function of the mammalian oviductal sperm reservoir. *J. Exp. Zool.* 292, 210–215.