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Distribution of extracellular matrix molecules in human uterine tubes during the menstrual cycle: a histological and immunohistochemical analysis

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Abstract

The uterine tube (UT) is an important and complex organ of the women's reproductive system. In general, the anatomy and basic histology of this organ are well-known. However, the composition and function of the extracellular matrix (ECM) of the UT is still poorly understood. The ECM is a complex supramolecular material produced by cells which is commonly restricted to the basement membrane and interstitial spaces. ECM molecules play not only a structural role, they are also important for cell growth, survival and differentiation in all tissues. In this context, the aim of this study was to evaluate the deposition and distribution of type I and III collagens and proteoglycans (decorin, biglycan, fibromodulin and versican) in human UT during the follicular and luteal phases by using histochemical and immunohistochemical techniques. Our results showed a broad synthesis of collagens (I and III) in the stroma of the UT. The analysis by regions showed, in the mucosa, a specific distribution of versican and fibromodulin in the epithelial surface, whereas decorin and fibromodulin were observed in the lamina propria. Versican and decorin were found in the stroma of the muscular layer, whereas all studied proteoglycans were identified in the serosa. Curiously, biglycan was restricted to the wall of the blood vessels of the serosa and muscular layers. Furthermore, there was an immunoreaction for collagens, decorin, versican and fibromodulin in the UT peripheral nerves. The differential distribution of these ECM molecules in the different layers of the UT could be related to specific structural and/or biomechanical functions needed for the oviductal transport, successful fertilization and early embryogenesis. However, further molecular studies under physiological and pathological conditions are still needed to elucidate the specific role of each molecule in the human UT.

Key words: collagens; extracelullar matrix; human uterine tubes; menstrual cycle; proteoglycans.

Introduction

The uterine tube (UT) is a tubular organ that connects the periovarian space with the uterus. The wall of the UT is composed of three layers: the mucosa, the muscular layer and the serosa (Bloom, 1995; Ross & Pawlina, 2010; Mills, 2012), where the extracellular matrix (ECM) plays an important structural and reproductive regulatory function (Diaz et al. 2012). The ECM is composed of several complex molecules and interstitial liquid. These components are classified into fibres (collagens, elastic and reticular) and nonfibrillar molecules (glycoproteins and proteoglycans). The ECM of organs is typically restricted to basic compartments: basement membranes and interstitial spaces. It corresponds to a multi-molecular material complex, which comprises a scaffold that organizes tissues, providing cellular microenvironments and regulating many cellular functions (Hay, 1991; Häcker et al. 2005).

Collagens are helical molecules with rod-like rigid structures ~ 300 nm long and 1.5 nm in diameter, which are

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capable of spontaneous fibril formation (Silver et al. 2003). The phenotypic consequences of mutations in fibrillar collagen genes (I, II, III and V) indicate that a major function of these proteins is to provide high tensile strength elements at the tissue level (Lanza et al. 2007). Probably, the degree of cross-linking, the length, and the diameter of the collagen fibril assembly all affect the viscoelastic properties of the oviduct. For example, mutations in *COLA1* or *COL1A2*, the human genes encoding the α 1 and α 2 subunits of fibrillar collagen I, cause osteogenesis imperfecta or the clinical form of Ehlers–Danlos syndrome, characterized by skin hyperextensibility and fragility, and joint hypermobility with or without bone abnormalities (Byers & Cole, 2002; Steinmann & Superti-Furga, 2002; Lanza et al. 2007).

On the other hand, fibromodulin, biglycan and decorin form a group of structurally and functionally related molecules that participate in the organization of the ECM and have important effects on cell behaviour (Hocking et al. 1998; McEwan et al. 2006). These molecules are called small leucine-rich proteoglycans (SLRPs) and they carry one or more glycosaminoglycan (GAG) chains of diverse chemical composition: chondroitin sulphate, dermatan sulphate or keratan sulphate (Hocking et al. 1998; Kreis, 1999). Several SLRPs have been shown to interact with different types of collagen (fibrillar and non-fibrillar) and to recognize different collagen sites (McEwan et al. 2006). The interactions of collagens with SLRPs may be critical in a number of biological processes, such as maintenance and assembly of the ECM during development, growth and wound healing (Hocking et al. 1998). Gene ablation experiments have shown that mice lacking these SLRPs have abnormalities of the collagen fibrillogenesis, leading to fragile skin, corneal opacity, osteoporosis or osteoarthritis (Danielson et al. 1997; Xu et al. 1998; Ameye et al. 2002).

In humans, there is very limited information about the ECM of the UT, and most of this evidence comes from classical histochemical studies (Schultka, 1980, 1981a,b; Schultka et al. 1986, 1989, 1993). The interaction between ECM molecules, growth factors and the adrenergic system are suggested as part of the complex paracrine/autocrine mechanism needed for successful fertilization and early embryogenesis (Einspanier et al. 2000). On the other hand, although the description of UT physiology and pathology is interesting, a correct characterization of its structure and molecular ECM composition of its different layers is still needed. This characterization could provide basic information to elucidate some pathophysiological conditions (such as tubal pregnancy or cancer) as well as to provide the structural and molecular bases for the development of tissue engineering-based strategies to repair or replace damaged UT. Furthermore, from the regenerative point of view, there is evidence that the UT is an important source of autologous multipotent stem cells, highlighting the potential usefulness of this material that is usually discarded after some surgical procedures (Jazedje et al. 2009; Wang et al. 2015).

The presence and possible functions of several ECM molecules have been studied in the uterus of human and other species (e.g. mice, rats, baboon) (Aplin et al. 1988; Fazleabas et al. 1997; Greca et al. 1998, 2000). The importance of these molecules (especially collagens and proteoglycans) lies in their key roles and remodelling during the normal cyclic functions of the uterus as well as during the preimplantation, implantation and pregnancy periods (San Martin & Zorn, 2003; San Martin et al. 2003b; Salgado et al. 2009). In this context, the metalloproteinases (MMP) and their endogenous inhibitors (TIMPs) have an important role in the regulation and restructuring of the ECM of the female reproductive system, including menstruation, ovulation, implantation and uterine (Polette et al. 1994; Hulboy et al. 1997; Novaro et al. 2002; Noguchi et al. 2003; Diaz et al. 2012). Recently, it has been demonstrated in humans by PCR and zymography that there is a differential expression of MMP and TIMPs in the mucosa of the UT throughout the menstrual cycle, suggesting a steroidal regulation (Diaz et al. 2012). Based on the differential distribution and remodelling of the ECM in the female reproductive system, a differential tissue distribution of collagens and proteoglycans in the human UT was hypothesized. The aim of this study was to perform a histological characterization of the UT focused on determining the tissue distribution and deposition of collagens (types I and III) and proteoglycans (decorin, biglycan, fibromodulin and versican) in the follicular and luteal phases of the human UT.

Material and methods

Human tissue collection

In this study, 10 human healthy UTs were obtained from patients undergoing voluntary surgical sterilization. Tissues were collected from the 'Servicio de Ginecología y Obstetricia, Hospital San José, Santiago, Chile' with informed consent. UTs used in this study came from fertile patients (25–45 years old) who were underwent the Pomeroy technique and were selected based on specific exclusion criteria (Table 1). In this study, the ampulla, which is well-preserved followed the Pomeroy technique, was used for histological analyses. The menstrual cycle from each patient was achieved with the clinical history and by determining the plasma levels of estradiol and

Table 1 Clinical exclusion criteria

Exclusion criteria
Used hormonal contraceptive methods within 3 months before surgery
Tubal disease
Endometriosis
Sexually transmitted infectious agents (*Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*)
Pelvic inflammatory disease
Heavy alcohol usage and tobacco or drug abuse

progesterone. Based on these results, seven patients corresponded to the follicular phase and three to the luteal phase of the menstrual cycle. Finally, this study and research project (Grant no. 021501) was approved by the ethics and biosafety committee of the 'Servicio de Salud Metropolitano Norte' and "Universidad de Santiago de Chile".

Histological evaluation

The UTs were rinsed in phosphate-buffered saline (PBS), fixed in 10% neutral buffered formalin, dehydrated, cleared, paraffin-embedded and sectioned at 5 μm thickness following previously described methods (Carriel et al. 2017b). General histological analyses were with haematoxylin/eosin (Panreac, Darmstadt, Germany). Furthermore, ECM molecules were studied by histochemical or immunohistochemical methods. The collagens were histochemically analysed by picrosirius (Sigma-Aldrich, Product number: 365548, USA) as described previously (Carriel et al. 2016, 2017a). The expression of type I and III collagens was identified by indirect immunohistochemistry. The presence of acid proteoglycans was determined with the Alcian blue (Panreac, Darmstadt, Germany) histochemical method, and the expression of SLPRs (decorin, biglycan and fibromodulin) and versican was determined by immunohistochemistry as described previously (Carriel et al. 2017c; Garcia-Martinez et al. 2017).

Immunohistochemistry

In this study, the slides were rehydrated and treated for immunohistochemistry following standardized procedures developed by our group (Godoy-Guzman et al. 2012; Carriel et al. 2017c; Garcia-Martinez et al. 2017). Briefly, all steps were performed in a humid chamber to avoid dehydration of the sections. The endogenous peroxidase activity was blocked with 3% (v/v) H_2O_2 (Panreac, Darmstadt, Germany) in PBS or methanol (VWR Chemicals, Leuven, Belgium) for 10 min. Each of the succeeding steps was followed by three rinses with PBS. Non-specific antibody reaction was blocked by incubating the slide with 1× casein solution (Vector, Burlingame, CA, USA) for 15 min followed by the incubation of the primary antibodies. All the technical information related to antibodies used is summarized in Table 2. After rinsing in PBS, all the sections were incubated for 30 min at room temperature with specific peroxidase conjugated secondary antibodies (Table 2). The antigen-antibody reaction was visualized using 3,3′-diaminobenzidine (DAB) peroxidase substrate kit SK-4100 (Vector) followed by a slight contrast with Harris' haematoxylin. These procedures were performed at the same time using the same conditions to ensure reproducibility of the results. In addition, for each immunohistochemical reaction, negative controls were used by omitting the primary antibody. Furthermore, human skin was used as positive external control for collagens and human placenta for proteoglycans (data not shown).

Results

General histology

In all samples, the ampulla is internally lined by a simple cylindrical epithelium followed by the lamina propria, which is made up of loose connective tissue (Fig. 1B). The mucosa has a large number of folds with abundant branches (Fig. 1A). The muscular layer is composed of smooth muscle tissue in a circular and longitudinal fascicular arrangement (Fig. 1E). The serosa is covered with a simple squamous epithelium under which there is a connective tissue (Fig. 1F). Furthermore, large blood vessels and peripheral nerve fascicles were observed in the serosa (Fig. 1F).

Distribution of collagens

The interstice of the mucosa, muscular and serosa was stained red with the picrosirius technique (Fig. 1A). At the level of the lamina propria of the folds, the collagen fibres were organized in a parallel manner (Fig. 1B). In addition, a

Table 2 Antibodies used for the immunohistochemical analysis.

Antibodies	Dilution/incubation	Pretreatment	References
Rabbit anti-collagen I polyclonal	1 : 200 Overnight	EDTA buffer pH 8, 25 min at 95 °C	Acris antibodies, Germany, Product number: R1038.
Rabbit anti-collagen III polyclonal	1 : 250 60 min	Citrate buffer pH 6, 25 min at 95 °C	ABCAM Cambridge, UK. Product number: ab7778.
Rabbit anti-biglycan polyclonal	1 : 100 60 min	Citrate buffer pH 6, 25 min at 95 °C	ABCAM Cambridge, UK. Product number: ab49701.
Rabbit anti- fibromodulin polyclonal (LF-150)	1 : 500 Overnight	Chondroitinase ABC 60 min at 37 °C	Dr. Larry Fisher (National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD, USA)
Rabbit anti-versican polyclonal	1 : 100 Overnight at 4 °C	Chondroitinase ABC 60 min at 37 °C	ABCAM Cambridge, UK. Product number: ab19345
Goat anti-decorin polyclonal	1 : 500 Overnight at 4 °C	Chondroitinase ABC 60 min at 37 °C	R&System Product number: AF143
Horse anti-goat IgG (peroxidase)	RTU	–	Vector Laboratories, CA, USA; cat. no. PI 9500
Horse anti-rabbit IgG (peroxidase)	RTU	–	Vector Laboratories, CA, USA; cat. no. MP-7401

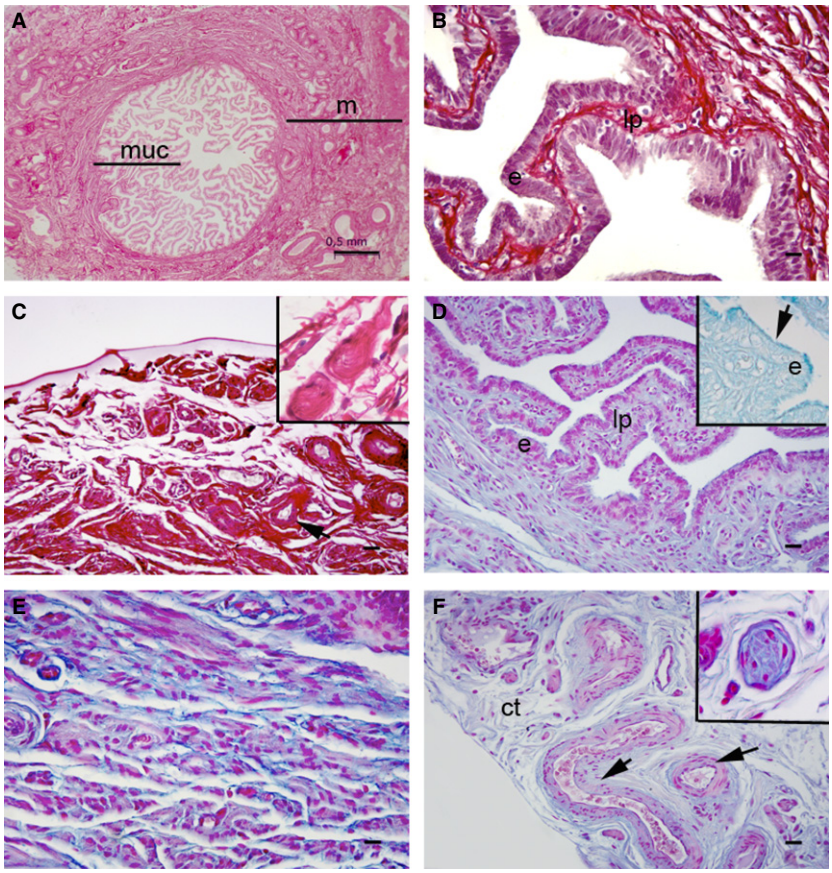

Fig. 1 Histological analysis of transversal section of UT. (A-C) Picrosirius histochemical staining. (D-F) Alcian Blue histochemical method. (A) Shows a broad distribution of collagen in the interstice of the mucosa (muc) and muscular layer (m). (B) Shows a mucosal fold covered by a simple cylindrical epithelium (e) and its subjacent lamina propria (lp) positive for collagen. (C) Serosa. Note the positive staining for collagen in the connective tissue, nerves (inset) and adventitia of large blood vessels (arrow). In the mucosa (D), notice a weak stain for Alcian blue in the lamina propria. The inset shows the coating epithelium of the folds whose apical surface is stained blue with Alcian blue. (E) Muscular layer. The connective tissue that surrounds the smooth muscle cells is positive for Alcian blue. (F) In the serosa, the connective tissue (ct), the adventitia of large blood vessels (arrows) and nerves (inset) are positive for Alcian blue. Scale bar: 20 μm.

positive reaction was observed in peripheral nerve fascicles and in the adventitia of blood vessels (Fig. 1F).

Collagen I and III were present and widely distributed in the interstice of the ampulla (Figs 2 and 3). In the mucosa there was positive immunostaining for both types of collagen in the lamina propria (Figs 2A,B and 3A,B). In the muscular layer the immunoreaction was distributed mainly at the level of the connective tissue that surrounds the smooth muscle cells and around the blood vessels (Figs 2C,D and 3C,D). In the serosa there was a positive immunoreaction in the connective tissue subjacent to the mesothelium (Figs 2E,F and 3E,F). Positive immunostaining was seen in the endoneurium and perineurium of peripheral nerves and around the blood vessels (Figs 2 and 3).

Distribution of proteoglycans

To obtain a general overview of most proteoglycans, Alcian blue staining was used. The apical surface of the epithelium was positive for Alcian blue staining, confirming the presence of acid mucopolysaccharides (Fig. 1D). The lamina propria showed a weak histochemical reaction with Alcian blue, whereas the smooth muscle tissue was surrounded by a positive reaction for proteoglycans (Fig. 1E). The connective tissue of the serosa was positive for Alcian blue staining, confirming the presence of acid proteoglycans at this level (Fig. 1F). Acid proteoglycans were also observed in the adventitia and tunica muscular of large blood vessels as well as in the endoneurium of peripheral nerve fascicles (Fig. 1F, inset).

Biglycan

The immunohistochemical analysis of biglycan revealed the specific distribution of this proteoglycan in the human UT, which was mainly associated to the adventitia and tunica muscularis of blood vessels of different calibres (Fig. 4). In the muscular layer and serosa, there was a positive expression of biglycan around the blood vessels (Fig. 4C,D) and in the connective tissue subjacent to the mesothelium (Fig. 4E, F). Curiously, biglycan was not found associated to peripheral nerves fascicles at this level.

Decorin

The immunohistochemical analysis of decorin showed a high distribution of this proteoglycans in the ampulla of the human UT. In the mucosa, there was positive immunostaining in the lamina propria of the folds (Fig. 5A,B). In the muscular layer the reaction was distributed mainly in the connective tissue surrounding the smooth muscle tissue (Fig. 5C,D). In the serosa there was a positive

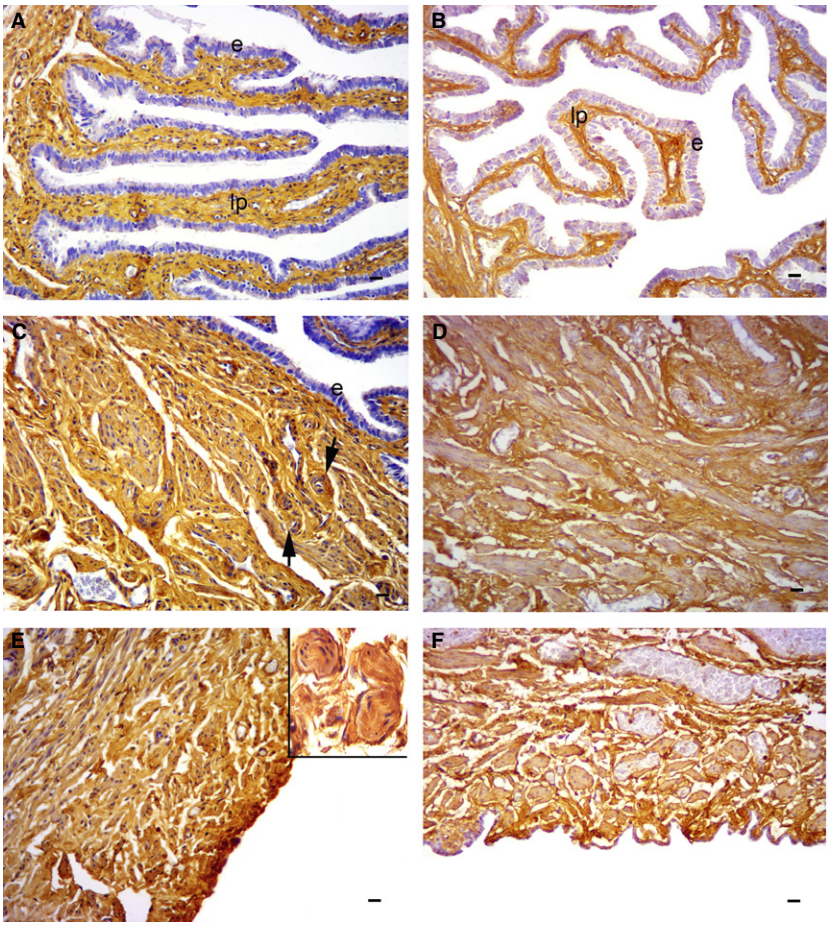

Fig. 2 Immunostaining of collagen I (brown stain) in UT sections. Immunostaining is shown in brown (DAB colorimetric reaction), whereas cell nuclei were contrasted with Harris' haematoxylin. (A,C,E) Follicular phase. (B,D,F) Luteal phase. (A,B) Show immunostaining, circumscribed mainly at the lamina propria (lp) level. (C,D) Show the distribution of collagen in the connective tissue of the muscular layer and around the blood vessels (arrows). (E,F) The serosa presents the distribution of collagen I in the connective tissue subjacent to the mesothelium. (E, Inset) Shows the positive immunostaining for collagen in nerves. E: epithelium. Scale bar: 20 μm.

immunoreaction in the connective tissue subjacent to the mesothelium (Fig. 5E,F). In relation to blood vessels, decorin was observed in the adventitia, whereas in the case of peripheral nerve fascicles, decorin was expressed in the endoneurium (Fig. 5).

Fibromodulin

The analysis of fibromodulin revealed positive immunostaining in the apical epithelial surface of the mucosa (Fig. 6A,B). Furthermore, fibromodulin was slightly expressed in the lamina propria. In the muscular layer and serosa, the reaction was mainly distributed in the connective tissue and smooth muscle cells (Fig. 6C,D,E,F). The analysis of blood vessel and peripheral nerves revealed a strong positive reaction in the adventitia and endoneurium, respectively (Fig. 6, insets).

Versican

The immunohistochemical analysis of versican proteoglycans showed a positive reaction in the apical epithelial surface part of the mucosa. This reaction was comparable to Alcian blue and fibromodulin, confirming the presence of these proteoglycans at this level (Fig. 7A,B). In the muscular layer, the immunoreaction was mainly distributed in the connective tissue that surrounds the smooth muscle tissue (Fig. 7C,D). In the serosa, versican was found in the connective tissue subjacent to the mesothelium, showing a comparable distribution with the other proteoglycans analysed (Fig. 7E,F). This analysis confirms the presence of versican in the tunica adventitia and tunica muscularis of blood vessels and in the endoneurium of peripheral nerve fascicles (Fig. 7, insets).

The results for each of the individual structures have been summarized in Table 3.

Discussion

In this study, we described the deposition and the specific tissue distribution of collagens (type I and III), some SLRPs (decorin, biglycan and fibromodulin) and versican PG in the human UT during the two phases of the menstrual cycle. The importance of these molecules lies in their key roles during the normal function of the uterus during implantation and pregnancy (San Martin & Zorn, 2003; San Martin et al. 2003a; Salgado et al. 2009). Furthermore, these molecules provide structure and biomechanical properties to most organs (Ushiki, 2002; Suki et al. 2005) and are important for tissue healing (Xue & Jackson, 2015).

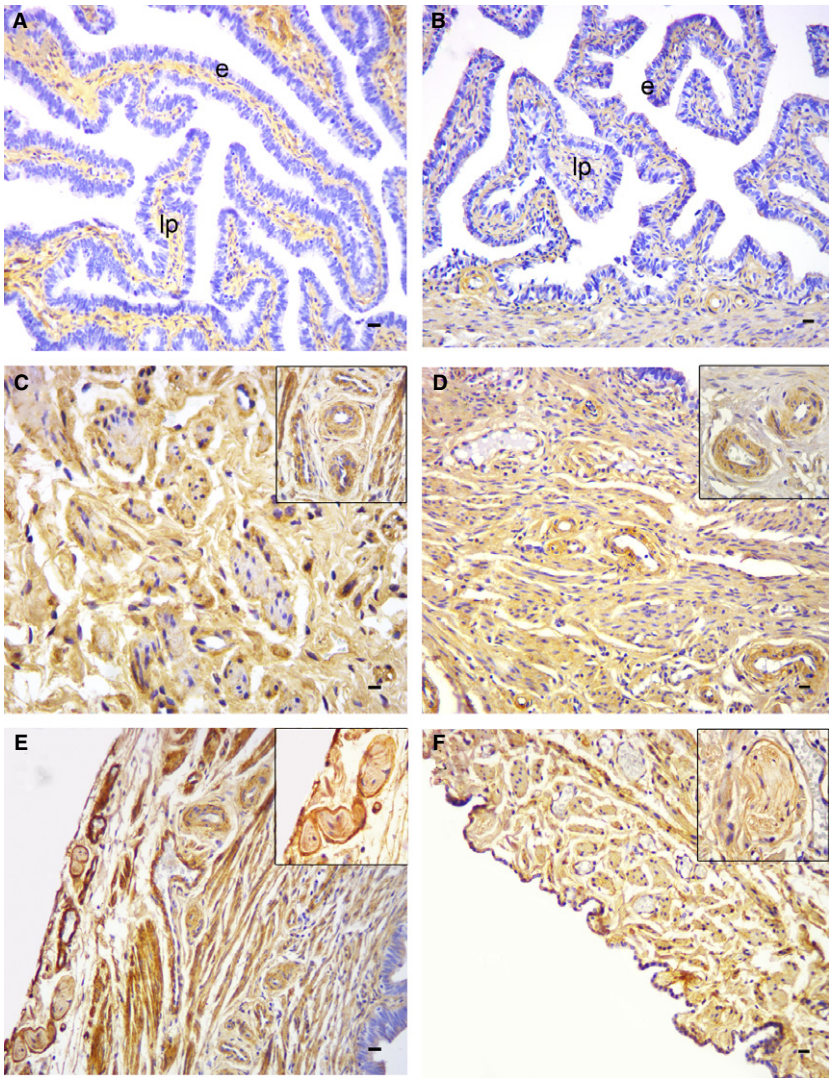

Fig. 3 Immunostaining of collagen III (brown stain) in UT sections, and counterstaining with haematoxylin. (A,C,E) Follicular phase. (B,D,F) Luteal phase. (A,B) Positive immunostaining in the lamina propria (lp) of the folds of mucosa. (C,D) Note the distribution of collagen in the connective tissue of the muscular layer and around the blood vessels (insets). (E,F) The serosa presents the distribution of collagen III in the connective tissue subjacent to the mesothelium. The insets show positive immunostaining for collagen III in the nerves. E: epithelium. Scale bar: 20 μm.

In general, all the connective tissues are composed of cells and ECM, which include water and a wide variety of biological macromolecules. One of the most important macromolecules for determining the biomechanical properties of a tissue are collagens (I, II and III) (Ushiki, 2002; Suki et al. 2005). In this context, we found a broad distribution of type I and III collagens in the ECM of the UT, an aspect that was demonstrated by the picrosirius staining and by immunohistochemistry. Our results confirmed the presence of type I and III collagens from the mucosa to the serosa of the UTs, especially collagen type I, which was more strongly stained than collagen type III. These results are similar to those described by Schultka et al. (1993), who studied the distribution of collagens (I, III, IV, V, VI) in oviducts (mucosa) of women between 32 and 67 years of age. The present authors conclude that the connective tissue of the UT is composed of important structural functional proteins, which form a complicated architecture of the ECM that changes due to the ageing process. Furthermore, Ohashi et al. (2003) demonstrated the expression of collagen I in the oviduct of Japanese quail by PCR and immunofluorescence. Probably, the broad distribution of type I and III collagens are needed for adequate structure and motility of the UT during the fertile period in humans. We did not find differences in the distribution of these collagens between follicular or luteal phases, supporting the structural role of these molecules.

It is well known that proteoglycans are involved in a large variety of biological processes, including structural maintenance, remodelling, cellular adhesion and signal transmission (Kreis, 1999; Giachini et al. 2008). In spite of extensive information on the expression and functions of proteoglycans in the reproductive organs (animal models), the exact role of these molecules in human reproductive organs has not been determined (Kitaya et al. 2012). In this sense, our study confirms the presence and differential expression of acid proteoglycans by the Alcian blue histochemical method and, more specifically, by immunohistochemistry.

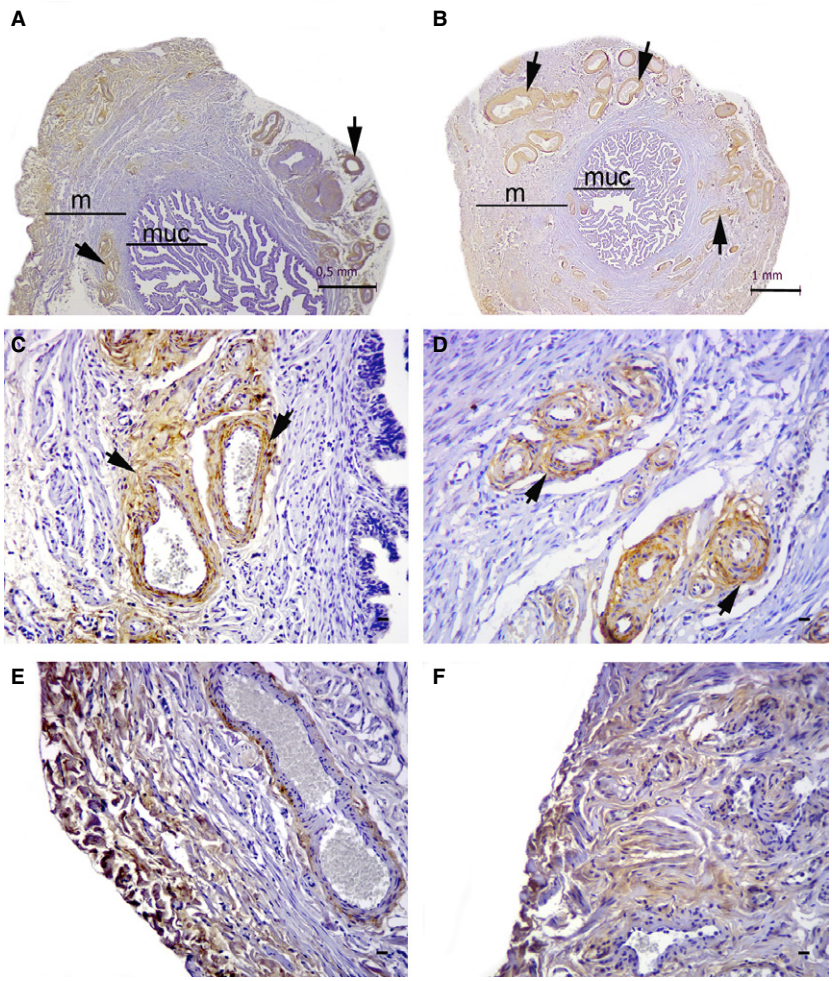

Fig. 4 Immunostaining of biglycan (brown stain) in UT sections, and counterstaining with haematoxylin. (A,C,E) Follicular phase. (B,D,F) Luteal phase. (A,B) Immunostaining circumscribed to different calibre blood vessels (arrows) of the muscular layer (m) and serosa. (C,D) Immunostaining circumscribed to the adventitious and muscular layers of blood vessels (arrows) in the muscular layer. (E,F) Serosa where there is a positive immunoreaction for biglycan in the connective tissue subjacent to the mesothelium. Muc: mucosa. Scale bar: 20 μm.

The Alcian blue histochemical method revealed a broad distribution of acid proteoglycans in the three histological layers of the human ampulla. Similar to other mucosa, Alcian blue was specifically positive in the apical surface of the epithelial cells, suggesting the synthesis of mucopolysaccharides by these cells, which is common in mucosa of reproductive organs (uterus and endocervix) (Mills, 2012).

In the case of biglycan, this SLRP is composed of a core protein and two chains of chondroitin or dermatan sulphate glycosaminoglycans (Kreis, 1999), which is highly distributed among different organs, such as uterus (San Martin et al. 2003a; Salgado et al. 2009), placenta (Giachini et al. 2008), human fetal lungs (Godoy-Guzman et al. 2012) and cartilage (Garcia-Martinez et al. 2017). The immunohistochemical analysis of biglycan in the human UTs demonstrated that this molecule was restricted to muscular and adventitia tunica of large and intermediate blood vessels. Additionally, a highly specific immunostaining of this molecule was observed in the ECM subjacent to the mesothelium in the serosa. This immunostaining pattern was comparable to the pattern observed in the human fetal lung blood vessels (Godoy-Guzman et al. 2012). Some *in vitro* studies support our findings and have described the expression of biglycan by endothelial cells and smooth muscles (Jarvelainen et al. 1991). Biglycan is classically associated to the fibrillogenesis of collagen fibres, showing a comparable tissue distribution to these molecules (Schonherr et al. 1995; San Martin & Zorn, 2003; San Martin et al. 2003a). Similar findings were observed in engineered elastic cartilage under culture conditions and in human elastic cartilage (Garcia-Martinez et al. 2017). Curiously, in this study the association between biglycan and collagens only occurred in the blood vessels and serosa, suggesting that biglycan could play a role in the structural organization of the collagen-rich ECM at these levels.

Decorin is a SLRP composed of a core protein linked to the glycosaminoglycan chains of chondroitin sulphate or dermatan sulphate (Cheng et al. 1994). Decorin has been described as a promiscuous molecule because it interacts with different ECM molecules, adhesion molecules and receptors (McEwan et al. 2006). Moreover, there is evidence that both decorin and biglycan bind to transforming growth factor β (TGF-β), modulating its activity as a mediator in growth inhibition (Yamaguchi et al. 1990; Hausser

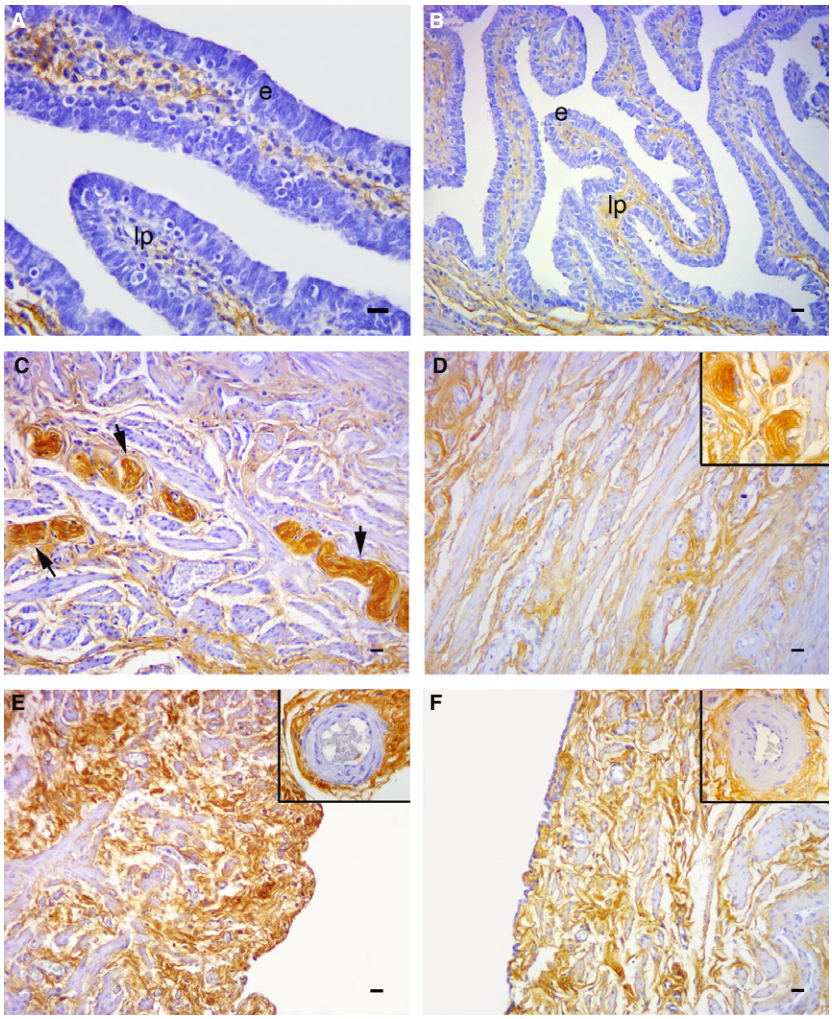

Fig. 5 Immunostaining of decorin (brown stain) in UT sections, and counterstaining with haematoxylin. (A,C,E) Follicular phase. (B,D,F) Luteal phase. (A,B) Positive immunostaining in the lamina propria (lp) of the folds of mucosa. (C,D) In the muscular layer, the reaction is circumscribed mainly to the level of the connective tissue that surrounds the smooth muscle cells. (C, inset D) Note the positive immunostaining for decorin in the nerves (arrows). (E,F) Positive immunoreaction in the connective tissue subjacent to the mesothelium. Moreover, there is a positive immunoreaction in the adventitia layer of large blood vessels (insets). E: epithelium. Scale bar: 20 μm.

et al. 1994; Kolb et al. 2001). Decorin is also associated to collagen fibres and it has been found to interact with type I and VI collagens, supporting the hypothesis that decorin may play a role in the ECM assembly (Bidanset et al. 1992; Scott et al. 2006). However, there is contradictory evidence concerning whether decorin supports the formation of thick or thin collagen fibres (Vogel et al. 1984; Uldbjerg & Danielsen, 1988; San Martin et al. 2003a). More recently, the *in vitro* synthesis of immature and poorly organized type I collagen fibres by mesenchymal stem cells into fibrin-agarose hydrogels was associated to a lack of decorin (Carriel et al. 2017c). Another *in vitro* study demonstrated that the incorporation of decorin during type I collagen hydrogel formation resulted in an increase and better organization of the collagen hydrogels (Reese et al. 2013). In this sense, both *in vitro* studies highlighted the importance of decorin in the type I collagen extracellular assembly. In this context, our results showed a similar distribution of type I collagen and decorin in the ECM that surrounds the smooth muscle bands of the muscular layer and the ECM subjacent to the mesothelium, where this pattern was also comparable to biglycan. Furthermore, decorin was strongly positive in the endoneurium of peripheral nerve fascicles. Curiously, decorin was negative in the tunica muscularis of blood vessels where biglycan was strongly positive and correlated to the pattern of type III collagen. In this context, it is possible that decorin could be related to the organization of type I collagen in the ECM of the UT and in the endoneurium of peripheral nerves, but not in the UT blood vessels.

Fibromodulin presents keratan sulphate and polylactosamine side chains, as well as clusters of tyrosine-sulphate residues at their N-termini. Fibromodulin binds to type I, II, VI and XI collagen in solid phase binding assays (Hedbom & Heinegard, 1993). Furthermore, it has been described to participate in the process of collagen fibrillogenesis and can act as a reservoir of growth factors (Vogel et al. 1984; Hocking et al. 1998; Salgado et al. 2009). Our results show a distribution of fibromodulin in the lamina propria (mucosa) and connective tissue of the muscular layer and serosa, and it may participate in the organization of collagen I, which has a similar distribution. Levens et al. (2005) state that

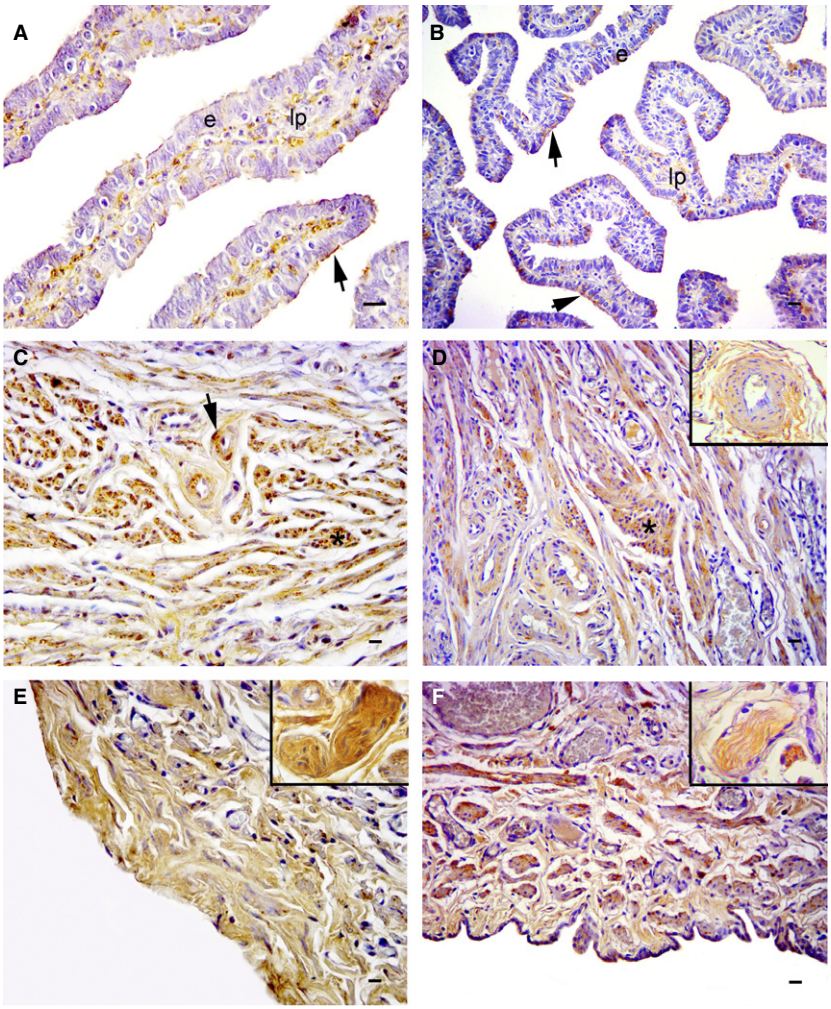

Fig. 6 Immunostaining of fibromodulin (brown stain) in UT sections, and counterstaining with haematoxylin. (A,C,E) Follicular phase. (B,D,F) Luteal phase. (A,B) Positive immunostaining on the apical epithelial surface (arrows) of the epithelium (e) and the lamina propria (lp). (C,D) In the muscular layer the reaction is circumscribed mainly to the level of the connective tissue and smooth muscle cells (asterisk). The arrow and inset correspond to a positive marking for fibromodulin around the blood vessels. (E, F) Positive immunoreaction in the connective tissue subjacent to the mesothelium. The insets correspond to a positive marking for fibromodulin in the nerves. Scale bar: 20 μm.

gonadotropin-releasing hormone affects the differential expression of fibromodulin in the myometrium of the proliferative and secreting phase of the human menstrual cycle, suggesting that this molecule is under the influence of the ovarian hormones.

Versican belongs to the family of hyaluronan-binding proteoglycans, constituting a gene family collectively termed 'hyalectins' (Margolis & Margolis, 1994). The highly interactive nature of versican provides a basis for its importance as a structural molecule, creating loose and hydrated matrices (Wight, 2002). Versican interacts with diverse molecules of the ECM and plays an important role in the assembly of the ECM (Matsumoto et al. 2006; Garcia-Martinez et al. 2017). Westergren-Thorsson et al. (1998) state that the structure of versican, having chondroitin/dermatan sulphated side chains and the ability to form large aggregates with hyaluronan, is of importance for immobilizing water and thereby increasing the swelling pressure between the collagen fibrils. *In vitro* observations indicate that versican inhibits the cell-substratum adhesion of primary fibroblasts to collagen I, fibronectin, vibronectin and laminin (Yamagata et al. 1993). In the UT, versican was distributed in the connective tissue that surrounds the smooth muscle cells of the muscular layer and serosa. Additionally, our study describes, for the first time, the presence of versican on the apical surface of the epithelial cells of the mucosa. This expression may be related to its cellular adhesion properties (Wight, 2002; Wu et al. 2002). However, there is no information related to the distribution and possible role of versican in the UT of laboratory animal or humans.

The synthesis of ECM molecules, such as collagen fibres, implies several multistep molecular and enzymatic processes that can be affected by specific mutations, resulting in genetic diseases and syndromes (Alberts et al. 2002). Mutations that affect the synthesis of collagen type I result in osteogenesis imperfecta, and mutations in genes that codify for collagens type II and III result in chondrodysplasias and Ehlers–Danlos syndrome, respectively. These genetic diseases directly affect the quality of life of many patients worldwide. In the case of Ehlers–Danlos syndrome, patients suffer connective tissue and vascular fragility, joint hypermobility and skin hyperextensibility (Byers & Cole, 2002; Steinmann & Superti-Furga, 2002; Lanza et al. 2007). In addition, the vascular type of this syndrome was associated

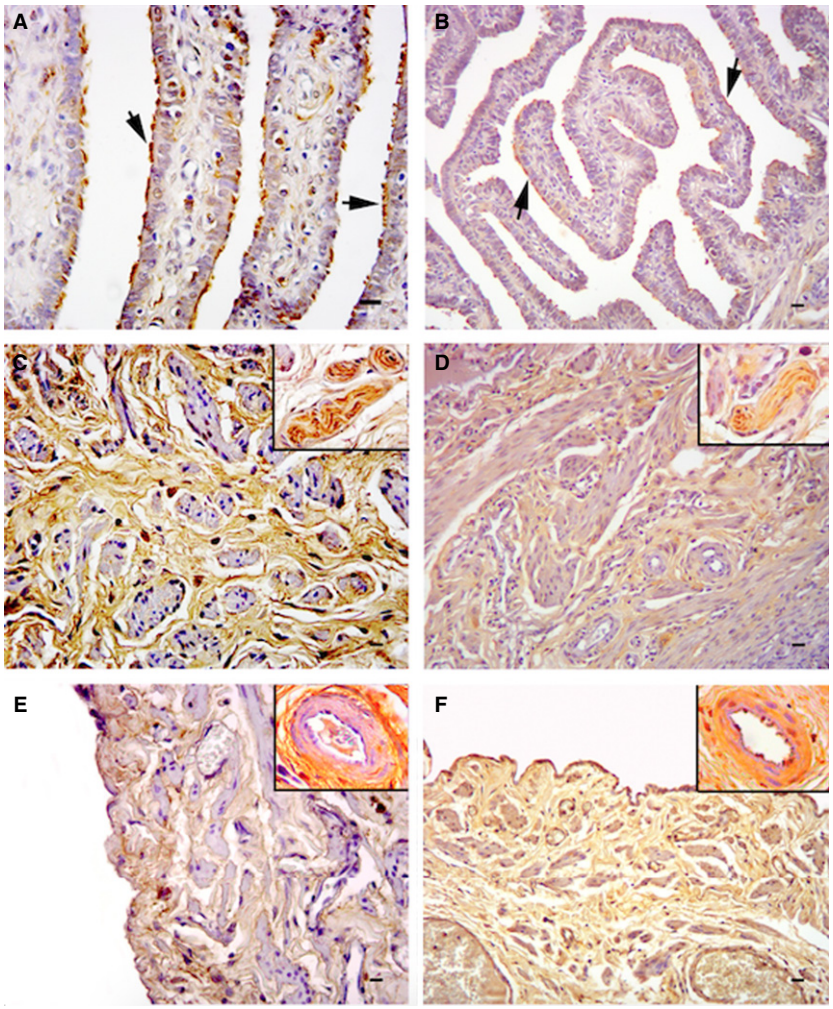

Fig. 7 Immunostaining of versican (brown stain) in UT sections, and counterstaining with hematoxylin. (A,C,E) Follicular phase. (B, D,F) Luteal phase. (A,B) Positive immunostaining on the apical epithelial surface (arrows) of the mucosa. (C,D) The reaction is distributed mainly at the level of the connective tissue that surrounds the smooth muscle cells. The insets correspond to a positive marking for versican in the nerves. (E,F) In the serosa there is a positive immunoreaction in the connective tissue subjacent to the mesothelium. The insets show positive immumostaining for versican in adventitia and tunica muscularis of blood vessels. Scale bar: 20 μm.

with several structural and functional effects on internal organs, such as colon, haemothorax (Alvarez et al. 2017) and rupture of the gravid uterus (Byers et al. 2017). However, there is no published evidence on the impact of ECM-associated mutation on the structure and function of human UT. In this context, gene ablation experiments could

Table 3 Collagen and proteoglycan distribution in different UT regions.

ECM molecules	Collagen I		Collagen III		Biglycan		Versican		Decorin		Fibromodulin	
Stage	F	L	F	L	F	L	F	L	F	L	F	L
Mucosa												
Apical portion of epithelium.	–	–	–	–	–	–	+	+	–	–	+	+
Lamina propria	+	+	+	+	–	–	–	–	+	+	+	+
Around the blood vessels	+	+	+	+	∓	∓	–	–	–	–	+	+
Muscular layer												
Connective tissue	+	+	+	+	–	–	+	+	+	+	+	+
Around the blood vessels	+	+	+	+	+	+	–	–	–	–	+	+
Smooth muscle	–	–	–	–	–	–	–	–	–	–	+	+
Serosa												
Around the blood vessels	+	+	+	+	+	+	–	–	–	–	+	+
Connective tissue	+	+	+	+	+	+	+	+	+	+	+	+
Nerves	+	+	+	+	–	–	+	+	+	+	+	+

FP, follicular phase; LP, luteal phase; (+), presence of the molecule; (–), absence of the molecule; (∓), Only some.

help to elucidate the function and possible effect of ECM molecules in UT.

Peripheral nerves are essential organs for the normal function of the UT and nerves fascicles can be easily observed in the serosa and muscular layer. Curiously, our study showed the expression of some ECM molecules that has not been described in these organs. Collagens type I and III were positive for Alcian blue (Mills, 2012). Here, for the first time, we described positive expression of decorin, fibromodulin and versican in the endoneurium of these small nerve fascicles, where biglycan was negative. In general, proteoglycans have been described as normal components of the central nervous system, where these molecules maintain the extracellular microenvironment (Hartmann & Maurer, 2001; Zimmermann & Dours-Zimmermann, 2008). In the case of peripheral nerves, the ECM plays crucial structural roles (Mills, 2012) but is also a key element in peripheral nerve regeneration (Carriel et al. 2013, 2014). Complete studies are needed to elucidate the role of decorin, fibromodulin, versican and other ECM molecules in native peripheral nerves and especially during regeneration.

In conclusion, our study provides new histological evidence of the deposition and tissue distribution of type I and III collagens and some collagen-related proteoglycans (decorin, biglycan, fibromodulin and versican) in the ECM of the human UT. The deposition and distribution of these molecules were consistent and unaffected by the phases of menstrual cycle. The differential deposition of these molecules in the different layers of the UT could provide structure and specific biomechanical properties needed for the oviductal transport process to enable successful fertilization and early embryogenesis. Future studies are still needed to elucidate the expression pattern and role of these molecules in specific pathological conditions. Furthermore, these results provide new insights for the development of regenerative strategies by tissue engineering based on the structure and ECM composition of the human UT.

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Conflict of interest

The authors declare no conflicts of interest.

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