Molecules involved in the sperm interaction in the human uterine tube: a histochemical and immunohistochemical approach

David Cajas,[1] Emanuel Guajardo,[2,3] Sergio Jara-Rosales,[4,5] Claudio Nuñez,[6] Renato Vargas,[6] Victor Carriel,[7] Antonio Campos,[7] Luis Milla,[1] Pedro Orihuela,[2] Carlos Godoy-Guzmán[1,8]

[1]Escuela de Medicina, Centro de Investigación Biomédica y Aplicada (CIBAP), Laboratorio de ingeniería de tejidos, Universidad de Santiago de Chile (USACH), Santiago, Chile
[2]Facultad de Química y Biología, Laboratorio de Inmunología de la Reproducción, Universidad de Santiago de Chile (USACH), Santiago, Chile
[3]Advanced Center for Chronic Diseases (ACCDiS), Facultad de Ciencias Químicas y Farmacéuticas and Facultad de Medicina, Universidad de Chile, Santiago, Chile
[4]Escuela de Obstetricia, Facultad de Ciencias para el Cuidado de la Salud, Universidad San Sebastián, Sede Los Leones, Santiago, Chile
[5]Programa de Doctorado en Enfermedades Crónicas, Universidad San Sebastián, Sede Los Leones, Chile
[6]Servicio de Ginecología y Obstetricia, Hospital San José, Santiago, Chile
[7]Department of Histology, Tissue Engineering Group, University of Granada, Spain; Instituto de Investigación Biosanitaria ibis.GRANADA, Granada, Spain
[8]Universidad de Santiago de Chile (USACH), Escuela de Medicina, Unidad de Histología, Santiago, Chile

ABSTRACT

In humans, even where millions of spermatozoa are deposited upon ejaculation in the vagina, only a few thousand enter the uterine tube (UT). Sperm transiently adhere to the epithelial cells lining the isthmus reservoir, and this interaction is essential in coordinating the availability of functional spermatozoa for fertilization. The binding of spermatozoa to the UT epithelium (mucosa) occurs due to interactions between cell-adhesion molecules on the cell surfaces of both the sperm and the epithelial cell. However, in humans, there is little information about the molecules involved. The aim of this study was to perform a histological characterization of the UT focused on determining the tissue distribution and deposition of some molecules associated with cell adhesion (F-spondin, galectin-9, osteopontin, integrin α_v/β_3) and UT's contractile activity (TNFα-R_1, TNFα-R_2) in the follicular and luteal phases. Our results showed the presence of galectin-9, F-spondin, osteopontin, integrin αV/β3, TNFα-R_1, and TNFα-R_2 in the epithelial cells in ampullar and isthmic segments during the menstrual cycle. Our results suggest that these molecules could form part of the sperm-UT interactions. Future studies will shed light on the specific role of each of the identified molecules.

Key words: human uterine tube; epithelium; menstrual cycle; immunohistochemistry; cell-adhesion molecules.

Correspondence: Prof. Dr. Carlos Godoy-Guzmán, Escuela de Medicina, Universidad de Santiago de Chile, Avda. Bdo. O'Higgins 3363, Correo 442, Santiago, Chile. Tel. +56.2.7183513. E-mail: carlos.godoy@usach.cl

Contributions: CGG, conceptualization and supervision of the review; DC, EG, experiments performing; SJR, CN, RV, provision of patients; CGG, PAO, LAM, VC, AC, participated in planning the experiments and contributed to drafting the manuscript. All the authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors have no conflict of interest to declare.

Ethics approval and consent to participate: the Ethics and Biosafety Committee of the Servicio de Salud Metropolitano Norte and the Universidad de Santiago de Chile approved this study (No102/carta No17/2019). Informed consent was obtained from each participant in this study.

Introduction

The uterine tube (UT), also called the Fallopian tube (or oviduct in animals), is the tubular organ connecting the periovarian space with the uterus.[1] Considering the tube in all its length, one can distinguish the fimbria, the ampulla, the isthmus and the intramural segment.[2] Its two major segments, the ampulla and the isthmus, differ both in structure and function.[2] The previously held belief that the UT were merely a passive conduit for the transportation of gametes and embryos during reproduction has been revised.[3] Today, it is widely recognized that the UT play a crucial role in various reproductive processes, the maturation and transport of gametes, fertilization, the early development of the embryo, and the formation of a sperm reservoir.[1,2,4-5]

In humans, millions of sperm cells are deposited in the vagina during ejaculation, but only a few thousand reach the UT.[5,7] Sperm have been observed to bind temporarily to the epithelial cells that line the caudal isthmus.[7] This interaction has been shown to extend the fertile lifespan of the sperm within the female reproductive tract.[7] In the isthmus region of the female reproductive tract, there exists a "functional spermatozoa reservoir" that serves to maintain a sufficient number of viable, potentially fertile sperm available for fertilization.[7-9] The presence of a sperm reservoir in the isthmus region of the female reproductive tract serves to facilitate the selection of competent sperm.[7,9] This process also modulates the capacitation of these sperm and ensures their release in controlled numbers, reducing the risk of polyspermy.[7,9]

The physical interactions that occur between spermatozoa and the epithelial cells lining the luminal surface of the UT may influence many aspects of sperm function.[11-15] These interactions are mediated by diverse cell-adhesion molecules located on the surface of both cell types. In this context, studies conducted in animal models revealed the existence of several molecules involved in these cell-cell interactions such as sialic acid-rich glycoproteins,[16] annexins,[17] fucose,[18] SBG,[20] Galβ1-3GalNAc,[19] galactose,[20] mannose,[21] osteopontin,[22] and integrins,[22] among others.[7,23] Studies have indicated that the adhesion process plays a critical role in the selection of high-quality spermatozoa and the preservation of their fertile life.[7,24-26] Several studies provide evidence that TNFα regulates the oviductal contractile activity.[27-29] Muro *et al*.[31] described that fluorescent spermatozoa moved back and forth together with peristaltic movement along the oviduct isthmus, suggesting that oviduct contractions may play a role in sperm migration. Thus, contractile activity in the isthmic could have a major influence on the binding or release between spermatozoa and epithelial cells, and migration through the UTs.[2,5,7,31] Furthermore, oviductal motile cilia are essential for oocyte pickup but dispensable for sperm and embryo transport.[32] The histological structure of the human UT has been studied;[1,3,33] however, more profound knowledge of normal UTs is necessary for a better understanding of pathological conditions. Around 30% of the infertile women worldwide have an associated UT pathology.[34,35] Studies have revealed that the interaction between human sperm and endosalpingeal tissue, which takes place *in vitro*, is disrupted in tissues obtained from women who have been diagnosed with endometriosis. This interaction is a vital component of the fertilization process and its disturbance in the presence of endometriosis has important implications for fertility and reproductive health.[36,37] Nevertheless, there is little information about the molecules present in both normal and pathological UTs that could be involved in these important biological interactions. Therefore, a histological description of these molecules could be vital to better understand and treat female infertility.

The aim of this study was to perform a histological characterization of the UT focused on determining the tissue distribution and deposition of some molecules associated with cell adhesion (F-spondin, galectin-9, osteopontin, integrin α_V/β_3) and UT's contractile activity (TNFα-R_1, TNFα-R_2) in the follicular and luteal phases.

Materials and Methods

Human tissue collection

The UTs were obtained exclusively from women undergoing surgical sterilization for reasons not related to this study. The tissues were collected in collaboration with the Servicio de Ginecología y Obstetricia of the Hospital San José, Santiago, Chile. The patients were fertile, aged 25 to 45 years, and voluntarily requested surgical sterilization. Table 1 summarizes the exclusion criteria. Menstrual cycle dating was determined using plasma levels of estradiol and progesterone together with the menstrual history. Seven women were in the follicular phase and three were in the luteal phase. The pieces of UTs removed by laparoscopy were ampullar and isthmic segments. The Ethics and Biosafety Committee of the Servicio de Salud Metropolitano Norte and the Universidad de Santiago de Chile approved this study (N°102/carta N°17/2019). Informed consent was obtained from each participant in this study.

Histological evaluation

UT samples, fixed in 10% neutral buffered formalin, were dehydrated in graded ethanol (70-100%) and embedded in paraffin for routine histology. Serial sections of 5 µm thickness were stained with hematoxylin/eosin for general histological assessment. Furthermore, an overview of the glycoproteins present in the UTs were identified by periodic acid-Schiff (PAS) histochemical stain (ScyTek Laboratories, Logan, UT, USA).[38,39] The distribution of acid proteoglycans and mucopolysaccharides was evaluated by the Alcian blue (Panreac, Darmstadt, Germany) histochemical method at pH 2.5.[1]

Immunohistochemistry

In this study, the slides were rehydrated and treated for immunohistochemistry following standardized procedures developed by our group.[1,40] All steps were performed in a humid chamber to prevent dehydration of the sections. Antigen retrieval was performed with citrate sodium solution (10 mM, pH 6.0) for 20 min at 95°C. Each of the succeeding steps was followed by three rinses with PBS. Non-specific antibody reaction was blocked by incubating the slides in PBS-T buffer with

Table 1. Exclusion criteria for patients in this work.

1.	Use of hormonal contraceptive methods within three months before surgery
2.	Endometriosis
3.	Tubal disease
4.	Pelvic inflammatory disease
5.	Sexually transmitted infection (*Chlamydia trachomatis* and/or *Neisseria* gonorrhoeae)
6.	Heavy alcohol usage and tobacco or drug abuse

2.5% (w/v) normal horse serum for 2 h at 25°C, followed by incubation of the primary antibodies. Table 2 summarizes technical information of the antibodies used. Endogenous peroxidase activity was blocked after primary antibody incubation by using 3% (v/v) H_2O_2 (Panreac) in PBS for 30 min. After rinsing in PBS, the slides were incubated for 1 h at room temperature with specific biotinylated pan-specific universal secondary antibody and one hour with streptavidin-peroxidase complex (Table 2). The antigen-antibody reaction was visualized using either 3,3′-diaminobenzidine (DAB) peroxidase substrate kit SK-4105 (Vector Laboratories, Burlingame, CA, USA) or NovaRED substrate kit SK-4805 (Vector), followed by a slight contrast with Harris' hematoxylin. These procedures were performed at the same time, using the same environmental conditions to ensure the reproducibility of the results. In addition, for each immunohistochemical reaction, negative technical controls were included by omitting the primary antibody and positive controls were used available (human placenta, human skin, between others).

Results

General histology

The UT is a tubular organ composed of three layers: mucosa, muscular layer, and serosa (Figure 1 A,B). The contours of the lumen of UT show longitudinal folds of the mucosa, which are more pronounced in the ampulla than isthmus. The muscular layer of the isthmus is thicker than the muscular layer of the ampulla. During the menstrual cycle, in the epithelium of all regions, three distinct cell types, ciliated, nonciliated, and basal cells, were distinguished (Figure 1 C-D). Ciliated cells have a spherical-shaped nucleus. Nonciliated cells have elongated nuclei. Basal cells have hyperchromatic nuclei and very pale cytoplasm (Figure 1 D). The epithelium of the UT is simple columnar. The luminal portion of the epithelium and basal lamina reacted with PAS stain, confirming the presence of glycoproteins (Figure 1E). The apical surface of the epithelium was positive for Alcian blue staining, confirming the presence of acid mucopolysaccharides (Figure 1F). It was determined that there were no differences in the distribution of PAS and Alcian Blue staining between the isthmus and ampulla segments throughout the menstrual cycle.

F-spondin

F-spondin exhibited intense staining in the epithelial cells of the mucosa (Figure 2). The cytoplasm of ciliated cells and secretory cells are positive for F-spondin. This analysis confirms the presence of F-spondin in the *tunica muscularis* of blood vessels of different caliber. No immunostaining was observed in the muscular layer. No difference was found in the expression or distribution of F-spondin between the two segments (ampulla and isthmus) during the menstrual cycle.

Galectin-9

The immunohistochemical analysis of galectin-9 showed a positive reaction in secretory cells and ciliated cells of the mucosa (Figure 3). This analysis confirms the presence of galectin-9 in the *tunica intima* and *muscularis* of blood vessels. No immunostaining was observed in the muscular layer. No difference was found in the expression or distribution of galectin-9 between the two segments (ampulla and isthmus) during the menstrual cycle.

Osteopontin

The analysis of osteopontin revealed positive immunostaining in the epithelial cells of the mucosa during the menstrual cycle (Figure 4). The analysis of blood vessels revealed a strong positive reaction in the *tunica muscularis*. There is no observable difference in osteopontin expression throughout the menstrual cycle or among the various segments of the UT (isthmus and ampulla).

Integrin α_V/β_3

The analysis of integrin α_V/β_3 revealed positive immunostaining in the epithelial cells of the mucosa during the menstrual cycle (Figure 5). In addition, a positive reaction was observed in the basal lamina of the mucosa's epithelium. The analysis of blood vessels revealed a strong positive reaction in the *tunica muscularis*. No difference was found in the expression or distribution of integrin α_V/β_3 between the two segments (ampulla and isthmus) during the menstrual cycle.

TNFα-R_2

TNFα-R_2 exhibited positive staining in the epithelial cells of the mucosa (Figure 6). The cytoplasm of ciliated cells and secretory cells are positive for TTNFα-R_2. This analysis confirms the weak presence of TNFα-R_2 in the *tunica muscularis* of blood vessels of different caliber. No immunostaining was observed in muscular layer. No differences were found in the expression or distribution of TNFα-R_2 between the two segments (ampulla and isthmus) during the menstrual cycle.

TNFα-R_2

The immunohistochemical analysis of TNFα-R_2 showed a positive reaction in secretory cells of mucosa (Figure 7). Although weak immunostaining was observed in ciliated cells. This analysis

Table 2. Antibodies and conjugates used for immunohistochemical analysis.

Antibody	Origin	Working dilution	References
Galectin-9	Rabbit polyclonal	1:75	Invitrogen, USA, product number PA5-32252
Osteopontin	Rabbit polyclonal	1:100	Invitrogen, USA, product number PA5-13494
F-spondin	Rabbit polyclonal	1:75	Santa Cruz Biotechnology, USA, product number sc-98924
Integrin α_V/β_3	Mouse monoclonal	1:10	Santa Cruz Biotechnology, USA, product number sc-7312
TNFα-R_1	Mouse monoclonal	1:25	Santa Cruz Biotechnology, USA, product number sc-8436
TNFα-R_2	Rabbit monoclonal	1:50	Santa Cruz Biotechnology, USA, product number sc-7862
Biotinylated Pan-specific universal antibody (anti-mouse/rabbit/goat IgG)	Horse polyclonal	RTU	Vector Laboratories, USA, product number PK-7800
Streptavidin-peroxidase complex	———————	RTU	Vector Laboratories, USA, product number PK-7800

confirms the presence of TNF-R_2 in the *tunica intima* and muscular layer of the blood vessels. No immunostaining was observed in muscular layers. No differences were found in the expression or distribution of TNFα-R_2 between the two segments (ampulla and isthmus) during the menstrual cycle. The results for each of the individual structures have been summarized in Table 3.

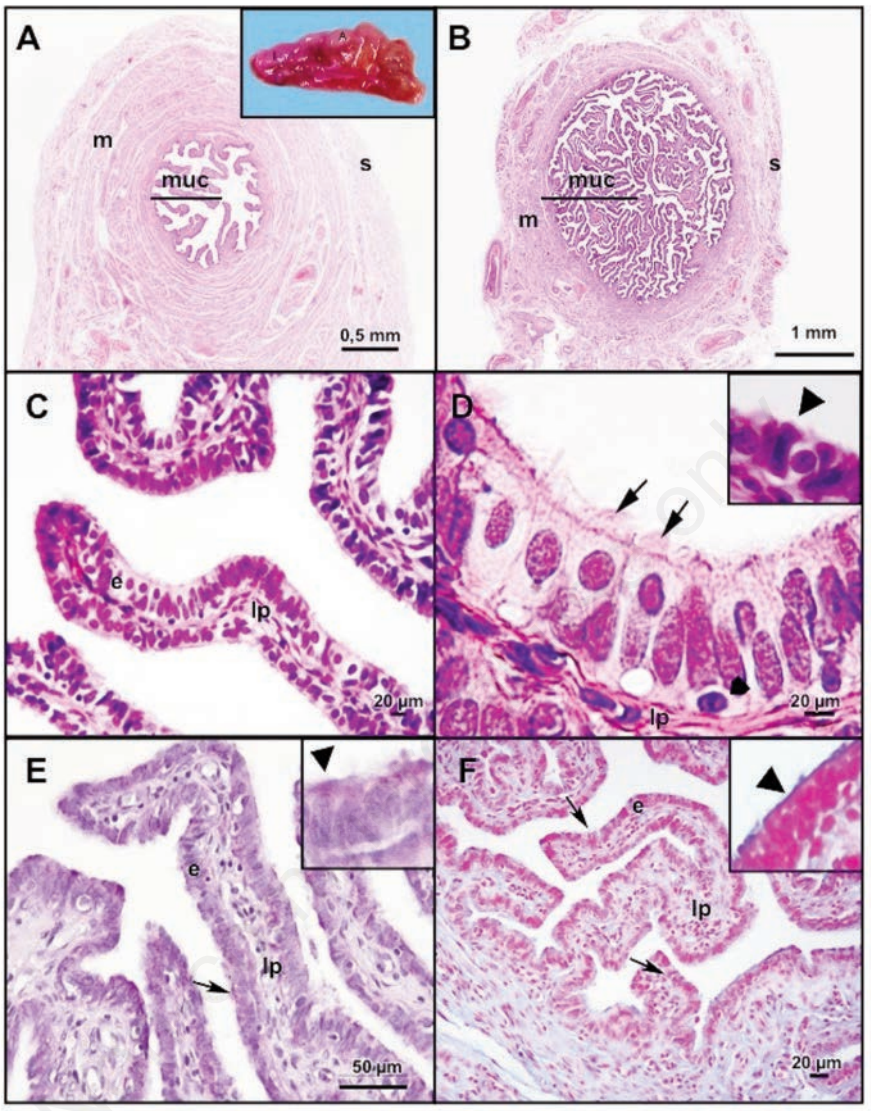

Figure 1. Histological analysis of UT cross section. A-D) Hematoxylin & Eosin stain. E) PAS stain. F) Alcian blue stain. Transversal section of isthmus (A) and ampulla (B). The UT is a tubular organ that connects the periovarian space with the uterus. A) Anatomical characteristics of UTs (inset); the ampulla has more mucosal folds than the isthmus. B) The muscular layer (m) of the isthmus is thicker than the muscular layer (m) of the ampulla. C) Shows a mucosal fold covered by a simple columnar epithelium (e) and its subjacent lamina propria (lp). D) Three different cell types were distinguished; ciliated (arrows), basal (arrowhead), and nonciliated cells (inset). E) The luminal portion of the epithelium (arrows) and basal lamina (arrowhead) reacted to PAS stain. F) The apical surface of the epithelium (arrows) was positive for Alcian blue staining. Muc, mucosa; M, muscular; S, serosa; I, isthmus; A, ampulla.

Table 3. Molecules distribution in mucosa of UTs during menstrual cycle.

	Galectin-9		F-spondin		Osteopontin		Integrin α_v/β_3		TNFα-R_1		TNFα-R_2	
Stage	F	L	F	L	F	L	F	L	F	L	F	L
Mucosa												
Ciliated cells	±	±	+	+	+	+	+	+	+	+	±	±
Secretory cells	+	+	+	+	+	+	+	+	+	+	+	+
Basal lamina	+	+	+	+	+	+	+	+	-	-		
Lamina propria	-	-	-	-	-	-	-	-	-	-	-	
Blood vessels	+	+	+	+	+	+	+	+	±	±	+	+

F, Follicular phase; L, luteal phase; +, presence of the molecule; -, absence of the molecule; ±, weak presence of the molecule.

Discussion

Sperm migration in the UTs depends on different factors:[41] sperm motility and hyperactivation, peristaltic movements, and oviductal flow. A universal feature of sperm migration in the female genital tract of mammals is the remarkable reduction of the number of cells that reach the site of fertilization in comparison with the total number inseminated. The biological importance of this phenomenon is the prevention of polyspermy.[42] Chang and Suarez[43] showed that mouse spermatozoa can attach to and detach from the epithelium of the oviduct isthmus, suggesting that spermatozoa may bind and unbind several times as they migrate through the oviduct. In this context, the results of the present study demonstrated the presence and distribution of galectin-9, F-spondin, osteopontin, and integrin α_V/β_3 in the human UTs during the follicular and luteal phases of the menstrual cycle. The importance of these molecules lies in their key roles during physical interactions between spermatozoa and UT epithelial cells.[22,44-48] Epithelial-bound spermatozoa also contribute to the formation of an isthmic sperm reservoir, thought to be important to organize available functional spermatozoa for fertilization.[8,9,36] Furthermore, several studies provide evidence that TNFα regulates the oviductal contractile activity, thus it could play a role in sperm migration.[27-29,49] In this sense, the deposition and distribution of TNFα-R_1 and TNFα-R_2 observed in the epithelial cells of the UTs could suggest that the contractile activity in the UTs could eventually influence the interaction between the sperm and epithelial cells, and subsequent migration through the UTs.[2,5,7,30,43]

The molecular components that are responsible for mediating sperm-UT adhesion remain largely unknown. Although, it appears that various species, including pigs and cattle, may share some similar mechanisms.[7,23] In general, adhesion is ensured by lectin-like molecules on the sperm rostral surface that can bind carbohydrates exposed on the apical membranes of oviductal cells in a species-specific manner.[7,16] Reeve *et al*.[37] postulated that the recognition between the amino acid sequence Arg-Gly-Asp (RGD) and integrin receptors may contribute to the interaction between sperm and the human endosalpinx in the isthmic region. Our histological and histochemical analyses showed that during the menstrual cycle, the epithelium of all regions is composed of three distinct cell types: ciliated, nonciliated, and basal cells. The apical surface of the epithelium was positive for Alcian blue staining, revealing the presence of acid mucopolysaccharides. Additionally, the PAS histochemical method revealed a positive stain in the apical surface and basal lamina of the epithelial cells. These observations suggest the synthesis of glycoprotein and acid mucopolysaccharides by epithelial cells. In addition, in a previous study,[1] the finding of versican and fibromodulin proteoglycan in the apical portion of the epithelium was reported. This proteoglycan, glycoprotein, and acid mucopolysaccharides distribution may be related to its cellular adhesion properties and probably to their potential interaction with gametes.[7,49,50]

Galectins are a class of β-galactoside binding proteins[51] that play several roles, such as regulation of cell growth, immunomodulation, apoptosis, and cell adhesion.[51-54] Popovic *et al*.[55] suggest galectin-9 as a novel human epithelial endometrial marker for mid- and late-secretory and decidual phases. In this regard, galectin-9

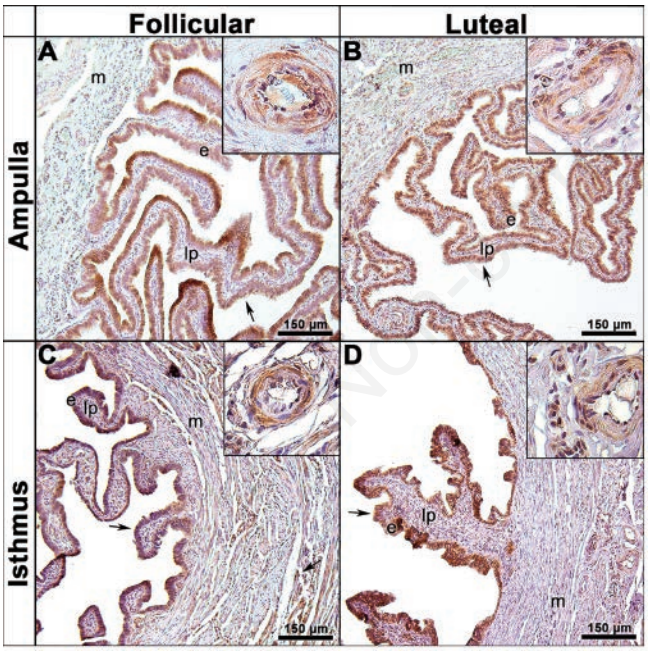

Figure 2. Immunostaining of F-spondin (brown stain) in UT sections. Immunostaining is shown in brown (DAB colorimetric reaction), whereas cell nuclei were contrasted with Harris' haematoxylin. A,C) Follicular phase. B,D) Luteal phase. A,B) Positive immunostaining in the ampulla's epithelium was observed (arrows); note the positive marking for F-spondin around the blood vessels (inset). C,D) Positive immunoreaction in the isthmus's epithelium was observed (arrows); note the positive marking for F-spondin around the blood vessels (inset). e, epithelium; lp, lamina propria; m, muscular layer.

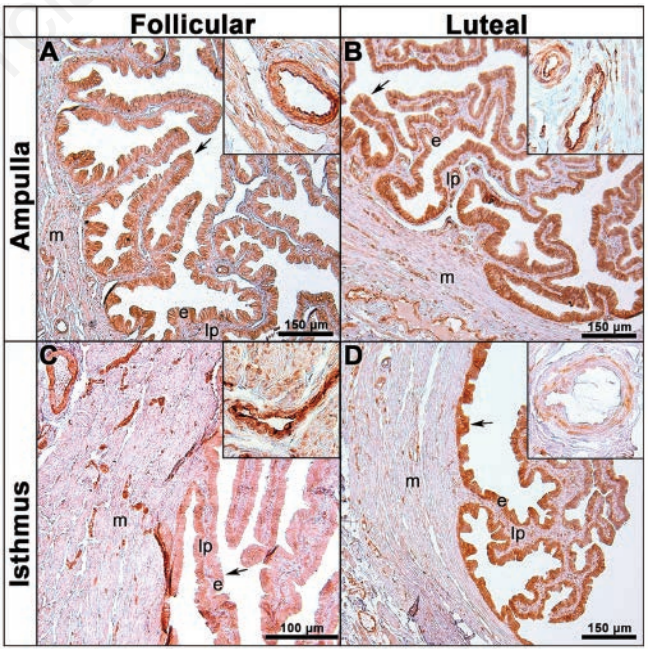

Figure 3. Immunostaining of galectin-9 (red stain) in UT sections. Immunostaining is shown in red (DAB colorimetric reaction), whereas cell nuclei were contrasted with Harris' haematoxylin. A,C) Follicular phase. B,D) Luteal phase. A,B) Galectin-9 was detected in the secretory cells and ciliated cells of the mucosa; note the positive marking for galectin-9 around the blood vessels (inset). C,D) Positive immunoreaction in the isthmus's secretory cells was observed; weak immunostaining was observed in the ciliated cells; note the positive marking for galectin-9 around the blood vessels (inset). e, epithelium; lp, lamina propria; m, muscular layer.

is a possible candidate for supporting the binding between endometrial epithelial cells and blastocysts. Furthermore, it has been described that galectins can affect cell adhesion both as agonist, as well as antagonist.[56] Galectins bind to cell adhesion molecules, such as fibronectin and laminin.[57] Fibronectin has previously been detected in ejaculated spermatozoa and spermatogenic cells.[58] Our results show a positive reaction in the secretory cells of UT's epithelium during the menstrual cycle. These results could be related to a different role for galectin-9 in the UT mucosa compared to the endometrium. In fact, it has been described that exposure of sperm to Gal-1 resulted in glycan-dependent modulation of the acrosome reaction, a key event in the fertilization process.[44] These studies, together with the presence of galectin-9 in the UT's epithelium, are consistent with the hypothesis that galectin-9 could interact with sperm during the maturation and capacitation processes. On the other hand, the analysis of blood vessels using immunohistochemistry confirmed the presence of galectin-9. Aanhane *et al*.[59] show that galectin-9 induced angiogenesis in the chick chorioallantoic membrane assay. In addition, O'Brien *et al*.[60] found a significant increase in blood vessel formation in response to galectin-9 in the matrigel plug angiogenesis assay, a murine model of angiogenesis. It is suggested that the distribution of galectin-9 in blood vessels is associated with its angiogenic properties.

Osteopontin is a highly phosphorylated glycophosphoprotein with acidic characteristics, rich in aspartic acid and N-terminal that includes an integrin-receptor binding zones.[61,62] In addition, integrin $\alpha_V\beta_1$, $\alpha_V\beta_5$ and $\alpha_9\beta_1$ act as receptors for osteopontin.[63-65] The integrin $\alpha_V\beta_3$ is primarily known to bind osteopontin *via* the RGD region.[66] Integrin β_1, β_3 and β_4 have been detected on the outer surface membrane and osteopontin in human spermatozoa.[67-69] The literature indicates that multiple osteopontin isoforms may play different roles in fertilization, early embryo development, and placentation.[22] Gabler *et al*.[22] showed that differential presence of osteopontin isoforms and integrins in the bovine oviduct indicates that osteopontin-integrin interactions have functional roles in normal oviduct physiology. In the present study, the analysis of osteopontin revealed positive immunostaining in the epithelial cells of UTs mucosa during follicular and luteal phases. As a result, our findings suggest that osteopontin found in the UTs epithelium may bind to integrins found in human spermatozoa. Additionally, the analysis of blood vessels revealed a strong positive reaction in the *tunica muscularis*. In this context, experimental evidence suggests that osteopontin may affect angiogenesis by acting directly on endothelial cells.[70,71]

F-spondin is an extracellular matrix protein that participates in the outgrowth of the neural tissue as well as in the inhibition of angiogenesis in the floor plate and paranotochordal area of the developing embryo.[72] In addition, F-spondin is expressed in the epithelial and stromal cells of mouse endometrium and Ishikawa cells, a human endometrial epithelial cell line.[45,46] In this context, it has been described that 2-methoxyoestradiol impairs mouse embryo implantation via activation of F-spondin in the mice uterus.[46] Curiously, F-spondin was identified as a new ovarian cancer marker.[73] The immunohistochemical analysis of F-spondin in the human UTs demonstrated that this molecule was restricted to ciliated cells and secretory cells. Furthermore, F-spondin immunostaining was positive in the epithelial cells of UTs mucosa and correlated to the pattern of type α_V/β_3. In this regard, some studies

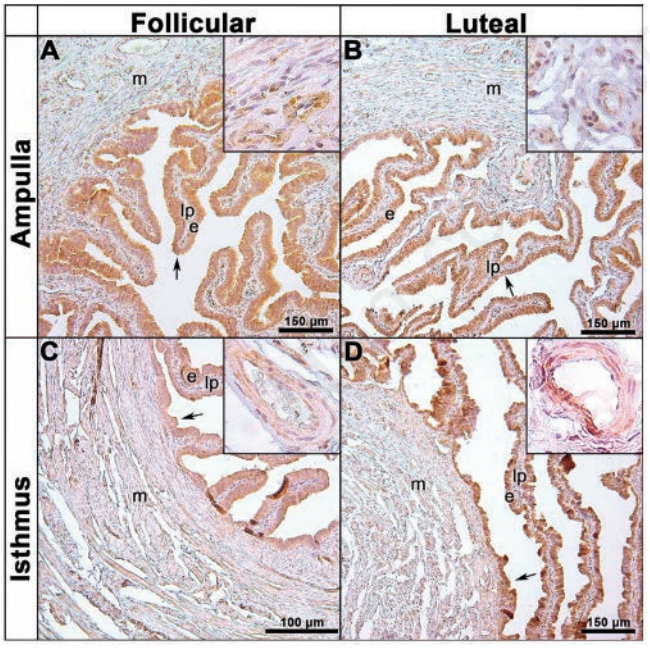

Figure 4. Immunostaining of osteopontin (brown stain) in UT sections. Immunostaining is shown in brown (DAB colorimetric reaction), whereas cell nuclei were contrasted with Harris' haematoxylin. A,C) Follicular phase. B,D) Luteal phase. A,B) Osteopontin was detected in the epithelial cells of the ampulla during the menstrual cycle; note the positive marking for osteopontin around the blood vessels (inset). C,D) Osteopontin was detected in the apical epithelial cells of the isthmus during the menstrual cycle; note the positive marking for osteopontin around the blood vessels (inset). e, epithelium; lp, lamina propria; m, muscular layer.

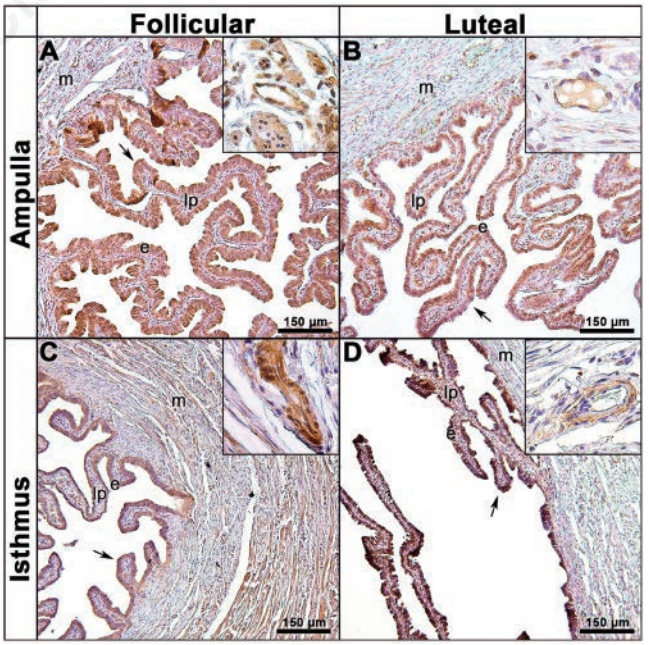

Figure 5. Immunostaining of integrin α_V/β_3 (brown stain) in UT sections. Immunostaining is shown in brown (DAB colorimetric reaction), whereas cell nuclei were contrasted with Harris' haematoxylin. A,C) Follicular phase. B,D) Luteal phase. A,B) Integrin α_V/β_3 was detected in the epithelial cells of the ampulla during the menstrual cycle; the analysis of blood vessels revealed a strong positive reaction in the *tunica muscularis* (inset); positive immunoreaction in the isthmus's epithelium was observed (arrows); note the positive marking for integrin α_V/β_3 around the blood vessels (inset). e, epithelium; lp, lamina propria; m, muscular layer.

reported that VSGP/F-spondin blockade α_V/β_3 on vascular endothelial cells.[74] This finding led us to suggest that F-spondin could be related to the anti-implantation effect in UTs.[45,46] Additionally, our study describes the presence of F-spondin in the *tunica muscularis* of blood vessels of different caliber. This expression may be related to its angiogenic properties because F-spondin is known to promote the growth of vascular smooth muscle cells.[75,76]

Integrins are alpha/beta heterodimeric adhesion glycoprotein receptors that regulate a wide variety of dynamic cellular processes such as cell migration, phagocytosis, growth, and development.[77] Furthermore, they provide a physical transmembrane link between the extracellular environment and the cytoskeleton, and are capable of transducing bidirectional signals across the cell membrane.[48] Additionally, $\alpha_v\beta_3$ is a receptor for proteins bearing an exposed Arg-Gly-Asp (RGD) tripeptide including vitronectin, fibronectin, fibrinogen, thrombospondin, osteopontin, von Willebrand factor, and some degraded laminins and collagens.[47] Several authors, have indicated that the endometrial epithelial expression of the integrin $\alpha_v\beta_3$, correlates with receptivity to the presenting embryo in humans.[78,79] Apparao *et al*.[80] showed in adhesion assays using Ishikawa cells that α_V/β_3 appears to be the primary receptor for osteopontin. These authors suggest that osteopontin and α_V/β_3 may play complementary roles during the endometrial implantation process. Our findings show integrin α_V/β_3 immunostaining in the epithelial surface cells of the mucosa during the menstrual cycle. Curiously, in this study, the association between α_V/β_3 and osteopontin in the UTs epithelium suggests that α_V/β_3 could play a role complementary to the interaction between spermatozoa and tubal epithelium. In addition, a positive reaction was observed in the basal lamina of mucosa's epithelium. These results are in agreement with previous reports stating that α_V/β_3 is a component of focal contact.[81] Analysis of blood vessels using immunohistochemistry confirmed the presence of α_V/β_3 in the *tunica muscularis*. Our results are consistent with previous studies that reported that α_V/β_3 antagonists could inhibit angiogenesis during development, wound healing, retinal neovascularization, and in growing tumors.[47]

Tumor necrosis factor-α (TNF-α) is a cytokine associated to the immune response in the female genital tract.[82] Several studies show that TNF-α could participate in reproductive functions, including fertilization, embryo development, and implantation.[82-86] The TNFα gene is expressed in mouse oviductal epithelial cells and human oviductal fluid contains TNFα protein.[87,88] Studies in human and animal models revealed that the embryo is capable of releasing TNFα.[28,89] Furthermore, the stage-specific expressions of mRNA for TNFα, TNFα-R_1, and TNFα-R_2 were detected in the bovine oviductal epithelial cells by RT-PCR and suggested the possible involvement of TNFα in the control of cyclic oviductal contraction.[27] Wijayagunawardane *et al*.[27] provided evidence that TNFα stimulates prostaglandins secretion and that up-regulation of the TNFα system occurs in the cow oviduct during the periovulatory period. In turn, prostaglandins play a major role in the oviductal transport of gametes/embryo as they stimulate muscular activity of the oviduct.[29,90] In this regard, contractile activity in the isthmus may have a significant influence on the distribution of spermatozoa relative to the time of ovulation.[2,5] In the present study, the analysis of TNFα-R_1 and TNFα-R_2 revealed positive immunostaining in the epithelial cells of the UTs mucosa during the menstrual cycle. These findings led us to suggest that contractile activity in

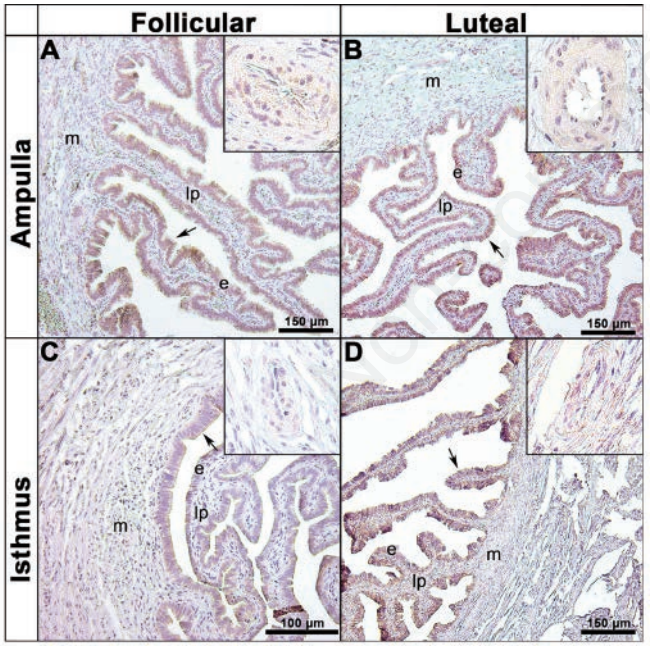

Figure 6. Immunostaining of TNFα-R_1 (brown stain) in UT sections. Immunostaining is shown in brown (DAB colorimetric reaction), whereas cell nuclei were contrasted with Harris' haematoxylin. A,C) Follicular phase. B,D) Luteal phase. A,B) TNFα-R_1 was detected in the epithelial cells of the ampulla during the menstrual cycle; note the weak immunostaining for TNFα-R_1 around the blood vessels (inset). C,D) TNFα-R_1 was detected in the epithelial cells of the isthmus during the menstrual cycle; note the weak immunostaining for TNFα-R_1 around the blood vessels (inset). e, epithelium; lp, lamina propria; m, muscular layer.

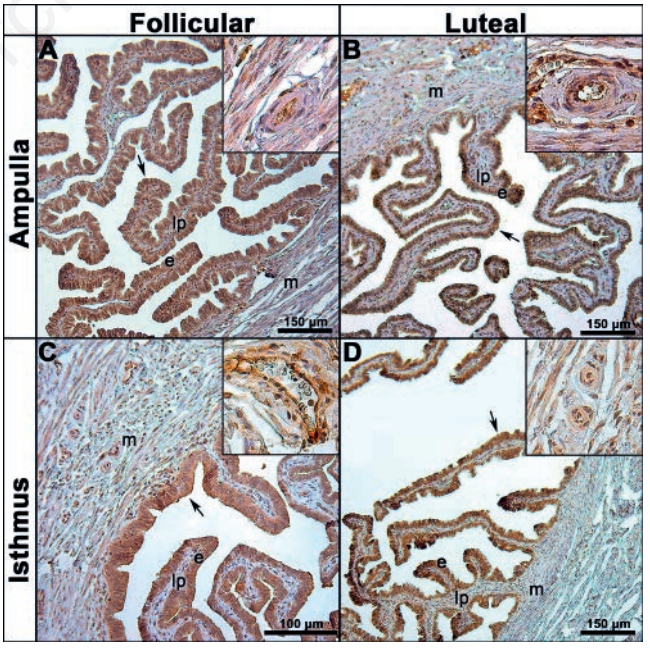

Figure 7. Immunostaining of TNFα-R_2 (brown stain) in UT sections. Immunostaining is shown in brown (DAB colorimetric reaction), whereas cell nuclei were contrasted with Harris' haematoxylin. A,C) Follicular phase. B,D) Luteal phase. A,B) TNFα-R_2 was detected in secretory cells of mucosa; weak immunostaining was observed in ciliated cells; note the positive immunostaining for TNFα-R_2 around the blood vessels (inset). C,D) Positive immunoreaction in the isthmus's secretory cells was observed; weak immunostaining was observed in ciliated cells; note the positive immunostaining for TNFα-R_2 around the blood vessels (inset). e, epithelium; lp, lamina propria; m, muscular layer.

the isthmic region could influence the binding or release of spermatozoa from epithelial cells and their migration through the UTs.[2,5,7,42,43,82] Interestingly, TNFα-R_1 and TNFα-R_2 immunostainings were positive in the epithelial cells of UTs mucosa and correlated to the pattern of osteopontin. In this regard, some studies reported that TNFα strongly induces osteopontin expression.[91,92] In conclusion, our comprehensive histological study demonstrates that UT epithelial cells produce galectin-9, F-spondin, osteopontin and α_V/β_3 integrin. In this sense, our results suggest that these molecules could form part of the sperm-UT interactions during the menstrual cycle. Additionally, an abundant distribution of TNFαR_1 and TNFα-R_2 was observed at the epithelial level, which could be related to the contractile activity of the UTs. Finally, the specific role of each of these molecules remains to be defined in future molecular and physiological studies.

Acknowledgments

The authors thank Veronica Yañez and Claudio Arriaza for assistance with the English in the manuscript and the technical support. This work was supported by the Universidad de Santiago de Chile (USACH), Agradecimientos Proyecto Dicyt 022201GG, Vicerrectoría de Investigación, Desarrollo e Innovación and the Tissue Engineering Group CTS-115, Department of Histology, University of Granada, Spain. ANID Nacional Becas/doctorado 21191519.

References

1. Godoy-Guzman C, Nunez C, Orihuela P, Campos A, Carriel V. Distribution of extracellular matrix molecules in human uterine tubes during the menstrual cycle: a histological and immunohistochemical analysis. J Anat 2018;233:73-85.
2. Croxatto HB. Physiology of gamete and embryo transport through the fallopian tube. Reprod Biomed Online 2002;4:160-9.
3. Godoy-Guzman C, Fuentes JL, Osses M, Toledo-Ordonez I, Orihuela P. The uterine tube: From herophilus to horacio croxatto. Int J Morphol 2018;36:387-90.
4. Gonzalez-Brusi L, Algarra B, Moros-Nicolas C, Izquierdo-Rico MJ, Aviles M, Jimenez-Movilla M. A comparative view on the oviductal environment during the periconception period. Biomolecules 2020;10:1690.
5. Hunter RHF. The fallopian tube. Their role in fertility and infertility. Cham: Springer; 1988.
6. Suarez SS, Pacey AA. Sperm transport in the female reproductive tract. Hum Reprod Update 2006;12:23-37.
7. Talevi R, Gualtieri R. Molecules involved in sperm-oviduct adhesion and release. Theriogenology 2010;73:796-801.
8. Hunter RHF. Human fertilization in vivo, with special reference to progression, storage and release of competent spermatozoa. Hum Reprod 1987;2:329-32.
9. Baillie HS, Pacey AA, Warren MA, Scudamore IW, Barratt CL. Greater numbers of human spermatozoa associate with endosalpingeal cells derived from the isthmus compared with those from the ampulla. Hum Reprod 1997;12:1985-92.
10. Hunter RHF. Ovarian endocrine control of sperm progression in the Fallopian tubes. Zygote 1994;2:363-6.
11. Yeung WS, Ng VK, Lau EY, Ho PC. Human oviductal cells and their conditioned medium maintain the motility and hyperactivation of human spermatozoa in vitro. Hum Reprod 1994;9:656-60.
12. Smith TT, Yanagimachi R. Capacitation status of hamster spermatozoa in the oviduct at various times after mating. J Reprod Fertil 1989;86:255-61.
13. Pollard JW, Plante C, King WA, Hansen PJ, Betteridge KJ, Suarez SS. Fertilizing capacity of bovine sperm may be maintained by binding of oviductal epithelial cells. Biol Reprod 1991;44:102-7.
14. Ellington JE, Ignotz GG, Ball BA, Meyers-Wallen VN, Currie WB. De novo protein synthesis by bovine uterine tube (oviduct) epithelial cells changes during co-culture with bull spermatozoa. Biol Reprod 1993;48:851-6.
15. Chian RC, Sirard MA. Fertilizing ability of bovine spermatozoa cocultured with oviduct epithelial cells. Biol Reprod 1995;52:156-62.
16. Cortes PP, Orihuela PA, Zuniga LM, Velasquez LA, Croxatto HB. Sperm binding to oviductal epithelial cells in the rat: role of sialic acid residues on the epithelial surface and sialic acid-binding sites on the sperm surface. Biol Reprod 2004;71:1262-9.
17. Teijeiro JM, Ignotz GG, Marini PE. Annexin A2 is involved in pig (Sus scrofa) sperm-oviduct interaction. Mol Reprod Dev 2009;76:334-41.
18. Ignotz GG, Cho MY, Suarez SS. Annexins are candidate oviductal receptors for bovine sperm surface proteins and thus may serve to hold bovine sperm in the oviductal reservoir. Biol Reprod 2007;77:906-13.
19. Marini PE, Cabada MO. One step purification and biochemical characterization of a spermatozoa-binding protein from porcine oviductal epithelial cells. Mol Reprod Dev 2003;66:383-90.
20. Carrasco LC, Romar R, Aviles M, Gadea J, Coy P. Determination of glycosidase activity in porcine oviductal fluid at the different phases of the estrous cycle. Reproduction 2008;136:833-42.
21. Ekhlasi-Hundrieser M, Gohr K, Wagner A, Tsolova M, Petrunkina A, Topfer-Petersen E. Spermadhesin AQN1 is a candidate receptor molecule involved in the formation of the oviductal sperm reservoir in the pig. Biol Reprod 2005;73:536-45.
22. Gabler C, Chapman DA, Killian GJ. Expression and presence of osteopontin and integrins in the bovine oviduct during the oestrous cycle. Reproduction 2003;126:721-9.
23. Rodriguez-Martinez H. Role of the oviduct in sperm capacitation. Theriogenology 2007;68:S138-S46.
24. Smith TT, Nothnick WB. Role of direct contact between spermatozoa and oviductal epithelial cells in maintaining rabbit sperm viability. Biol Reprod 1997;56:83-9.
25. Murray SC, Smith TT. Sperm interaction with fallopian tube apical membrane enhances sperm motility and delays capacitation. Fertil Steril 1997;68:351-7.
26. Dobrinski I, Smith TT, Suarez SS, Ball BA. Membrane contact with oviductal epithelium modulates the intracellular calcium concentration of equine spermatozoa in vitro. Biol Reprod 1997;56:861-9.
27. Wijayagunawardane MP, Gabler C, Killian G, Miyamoto A. Tumor necrosis factor alpha in the bovine oviduct during the estrous cycle: messenger RNA expression and effect on secretion of prostaglandins, endothelin-1, and angiotensin II. Biol Reprod 2003;69:1341-6.
28. Wijayagunawardane MP, Miyamoto A. Tumor necrosis factor alpha system in the bovine oviduct: a possible mechanism for embryo transport. J Reprod Dev 2004;50:57-62.
29. Spilman CH, Harper MJ. Effects of prostaglandins on oviductal motility and egg transport. Gynecol Invest 1975;6:186-205.
30. Muro Y, Hasuwa H, Isotani A, Miyata H, Yamagata K, Ikawa M, et al. Behavior of mouse spermatozoa in the female reproductive tract from soon after mating to the beginning of fertil-

ization. Biol Reprod 2016;94:80.
31. Chang H, Suarez SS. Unexpected flagellar movement patterns and epithelial binding behavior of mouse sperm in the oviduct. Biol Reprod 2012;86:1-8.
32. Yuan S, Wang Z, Peng H, Ward SM, Hennig GW, Zheng H, et al. Oviductal motile cilia are essential for oocyte pickup but dispensable for sperm and embryo transport. Proc Natl Acad Sci USA 2021;118:e2102940118.
33. Patek E. The epithelium of the human Fallopian tube. A surface ultrastructural and cytochemical study. Acta Obstet Gynecol Scand Suppl 1974;31:1-28.
34. Briceag I, Costache A, Purcarea VL, Cergan R, Dumitru M, Briceag I, et al. Fallopian tubes--literature review of anatomy and etiology in female infertility. J Med Life 2015;8:129-31.
35. Schlegel P, Fauser B, Carrel D, Racowsky C. Biennial review of infertility. New York: Springer; 2013.
36. Reeve L, Lashen H, Pacey AA. Endometriosis affects sperm-endosalpingeal interactions. Hum Reprod 2005;20:448-51.
37. Reeve L, Ledger WL, Pacey AA. Does the Arg-Gly-Asp (RGD) adhesion sequence play a role in mediating sperm interaction with the human endosalpinx? Hum Reprod 2003;18: 1461-8.
38. Suvarna SK, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. Oxford: Churchill Livingstone; 2012.
39. Vela-Romera A, Carriel V, Martin-Piedra MA, Aneiros-Fernandez J, Campos F, Chato-Astrain J, et al. Characterization of the human ridged and non-ridged skin: a comprehensive histological, histochemical and immunohistochemical analysis. Histochem Cell Biol 2019;151:57-73.
40. Pereda J, Sulz L, San Martin S, Godoy-Guzman C. The human lung during the embryonic period: vasculogenesis and primitive erythroblasts circulation. J Anat 2013;222:487-94.
41. Fujihara Y, Miyata H, Ikawa M. Factors controlling sperm migration through the oviduct revealed by gene-modified mouse models. Exp Anim 2018;67:91-104.
42. Orihuela PA, Ortiz ME, Croxatto HB. Sperm migration into and through the oviduct following artificial insemination at different stages of the estrous cycle in the rat. Biol Reprod 1999;60:908-13.
43. Chang HX, Suarez SS. Unexpected flagellar movement patterns and epithelial binding behavior of mouse sperm in the oviduct. Biol Reprod 2012;86:140.
44. Vasen G, Battistone MA, Croci DO, Brukman NG, Weigel Munoz M, Stupirski JC, et al. The galectin-1-glycan axis controls sperm fertilizing capacity by regulating sperm motility and membrane hyperpolarization. FASEB J 2015;29:4189-200.
45. Rincon-Rodriguez RJ, Orostica ML, Diaz P, Reuquen P, Cardenas H, Orihuela PA. Changes in the gene expression pattern induced by 2-methoxyestradiol in the mouse uterus. Endocrine 2013;44:773-83.
46. Guajardo-Correa E, Mena-Silva D, Diaz P, Godoy-Guzman C, Cardenas H, Orihuela PA. 2-Methoxyoestradiol impairs mouse embryo implantation via F-spondin. Reprod Fertil Dev 2019;31:689-97.
47. Nisato RE, Tille JC, Jonczyk A, Goodman SL, Pepper MS. alphav beta 3 and alphav beta 5 integrin antagonists inhibit angiogenesis in vitro. Angiogenesis 2003;6:105-19.
48. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002;110:673-87.
49. Wu TCJ, Lee SM, Jih MH, Liu JT, Wan YJY. Differential distribution of glycoconjugates in human reproductive-tract. Fertil Steril 1993;59:60-4.
50. Wight TN. Versican: a versatile extracellular matrix proteoglycan in cell biology. Curr Opin Cell Biol 2002;14:617-23.
51. Rabinovich GA. Galectins: an evolutionarily conserved family of animal lectins with multifunctional properties; a trip from the gene to clinical therapy. Cell Death Differ 1999;6:711-21.
52. Zick Y, Eisenstein M, Goren RA, Hadari YR, Levy Y, Ronen D. Role of galectin-8 as a modulator of cell adhesion and cell growth. Glycoconj J 2002;19:517-26.
53. Gray CA, Adelson DL, Bazer FW, Burghardt RC, Meeusen EN, Spencer TE. Discovery and characterization of an epithelial-specific galectin in the endometrium that forms crystals in the trophectoderm. Proc Natl Acad Sci USA 2004;101:7982-7.
54. Lahm H, Andre S, Hoeflich A, Kaltner H, Siebert HC, Sordat B, et al. Tumor galectinology: insights into the complex network of a family of endogenous lectins. Glycoconj J 2004;20:227-38.
55. Popovici RM, Krause MS, Germeyer A, Strowitzki T, von Wolff M. Galectin-9: a new endometrial epithelial marker for the mid- and late-secretory and decidual phases in humans. J Clin Endocrinol Metab 2005;90:6170-6.
56. Hughes RC. Galectins as modulators of cell adhesion. Biochimie 2001;83:667-76.
57. Kuwabara I, Liu FT. Galectin-3 promotes adhesion of human neutrophils to laminin. J Immunol 1996;156:3939-44.
58. Glander HJ, Schaller J, Rohwedder A, Henkel R. Adhesion molecules and matrix proteins on human spermatozoa. Andrologia 1998;30:289-96.
59. Aanhane E, Schulkens IA, Heusschen R, Castricum K, Leffler H, Griffioen AW, et al. Different angioregulatory activity of monovalent galectin-9 isoforms. Angiogenesis 2018;21:545-55.
60. O'Brien MJ, Shu Q, Stinson WA, Tsou PS, Ruth JH, Isozaki T, et al. A unique role for galectin-9 in angiogenesis and inflammatory arthritis. Arthritis Res Ther 2018;20:31.
61. Icer MA, Gezmen-Karadag M. The multiple functions and mechanisms of osteopontin. Clin Biochem 2018;59:17-24.
62. Standal T, Hjorth-Hansen H, Rasmussen T, Dahl IM, Lenhoff S, Brenne AT, et al. Osteopontin is an adhesive factor for myeloma cells and is found in increased levels in plasma from patients with multiple myeloma. Haematologica 2004;89:174-82.
63. Smith LL, Cheung HK, Ling LE, Chen J, Sheppard D, Pytela R, et al. Osteopontin N-terminal domain contains a cryptic adhesive sequence recognized by alpha9beta1 integrin. J Biol Chem 1996;271:28485-91.
64. Hu DD, Lin EC, Kovach NL, Hoyer JR, Smith JW. A biochemical characterization of the binding of osteopontin to integrins alpha v beta 1 and alpha v beta 5. J Biol Chem 1995;270: 26232-8.
65. Liaw L, Skinner MP, Raines EW, Ross R, Cheresh DA, Schwartz SM, et al. The adhesive and migratory effects of osteopontin are mediated via distinct cell surface integrins. Role of alpha v beta 3 in smooth muscle cell migration to osteopontin in vitro. J Clin Invest 1995;95:713-24.
65. Rodan GA. Osteopontin overview. Ann N Y Acad Sci 1995; 760:1-5.
67. Glander HJ, Schaller J. Beta 1-integrins of spermatozoa: a flow cytophotometric analysis. Int J Androl 1993;16:105-11.
68. Rohwedder A, Liedigk O, Schaller J, Glander HJ, Werchau H. Detection of mRNA transcripts of beta 1 integrins in ejaculated human spermatozoa by nested reverse transcription-polymerase chain reaction. Mol Hum Reprod 1996;2:499-505.
69. Schaller J, Glander HJ, Dethloff J. Evidence of beta 1 integrins and fibronectin on spermatogenic cells in human testis. Hum Reprod 1993;8:1873-8.
70. Dai J, Peng L, Fan K, Wang H, Wei R, Ji G, et al. Osteopontin induces angiogenesis through activation of PI3K/AKT and ERK1/2 in endothelial cells. Oncogene 2009;28:3412-22.
71. Shijubo N, Kojima H, Nagata M, Ohchi T, Suzuki A, Abe S, et

al. Tumor angiogenesis of non-small cell lung cancer. Microsc Res Tech 2003;60:186-98.
72. Feinstein Y, Klar A. The neuronal class 2 TSR proteins F-spondin and Mindin: a small family with divergent biological activities. Int J Biochem Cell Biol 2004;36:975-80.
73. Pyle-Chenault RA, Stolk JA, Molesh DA, Boyle-Harlan D, McNeill PD, Repasky EA, et al. VSGP/F-spondin: a new ovarian cancer marker. Tumour Biol 2005;26:245-57.
74. Terai Y, Abe M, Miyamoto K, Koike M, Yamasaki M, Ueda M, et al. Vascular smooth muscle cell growth-promoting factor/F-spondin inhibits angiogenesis via the blockade of integrin alphavbeta3 on vascular endothelial cells. J Cell Physiol 2001;188:394-402.
75. Miyamoto K, Morishita Y, Yamazaki M, Minamino N, Kangawa K, Matsuo H, et al. Isolation and characterization of vascular smooth muscle cell growth promoting factor from bovine ovarian follicular fluid and its cDNA cloning from bovine and human ovary. Arch Biochem Biophys 2001;390: 93-100.
76. Ohnuma K, Kaneko H, Noguchi J, Kikuchi K, Ozawa M, Hasegawa Y. Isolation and identification of F-spondin in the boar testis and its production during testis growth. J Reprod Dev 2007;53:151-8.
77. Arnaout MA, Goodman SL, Xiong JP. Structure and mechanics of integrin-based cell adhesion. Curr Opin Cell Biol 2007;19:495-507.
78. Lessey BA. Endometrial integrins and the establishment of uterine receptivity. Hum Reprod 1998;13S247-58; discussion 59-61.
79. Lessey BA, Castelbaum AJ, Sawin SW, Sun JH. Integrins as markers of uterine receptivity in women with primary unexplained infertility. Fertil Steril 1995;63:535-42.
80. Apparao KB, Murray MJ, Fritz MA, Meyer WR, Chambers AF, Truong PR, et al. Osteopontin and its receptor alphavbeta(3) integrin are coexpressed in the human endometrium during the menstrual cycle but regulated differentially. J Clin Endocrinol Metab 2001;86:4991-5000.
81. Burridge K, Chrzanowska-Wodnicka M, Zhong C. Focal adhesion assembly. Trends Cell Biol 1997;7:342-7.
82. Orostica ML, Zuniga LM, Utz D, Parada-Bustamante A, Velasquez LA, Cardenas H, et al. Tumour necrosis factor-alpha is the signal induced by mating to shutdown a 2-methoxyestradiol nongenomic action necessary to accelerate oviductal egg transport in the rat. Reproduction 2013;145:109-17.
83. Choo KB, Hsu MC, Tsai YH, Lin WY, Huang CJ. Nuclear factor kappa B and tumor necrosis factor-alpha modulation of transcription of the mouse testis- and pre-implantation development-specific Rnf33/Trim60 gene. FEBS J 2011;278:837-50.
84. Lee KS, Joo BS, Na YJ, Yoon MS, Choi OH, Kim WW. Relationships between concentrations of tumor necrosis factor-alpha and nitric oxide in follicular fluid and oocyte quality. J Assist Reprod Gen 2000;17:222-8.
85. Torchinsky A, Shepshelovich J, Orenstein H, Zaslavsky Z, Savion S, Carp H, et al. TNF-alpha protects embryos exposed to developmental toxicants. Am J Reprod Immunol 2003;49: 159-68.
86. Abdo M, Hisheh S, Arfuso F, Dharmarajan A. The expression of tumor necrosis factor-alpha, its receptors and steroidogenic acute regulatory protein during corpus luteum regression. Reprod Biol Endocrinol 2008;6:50.
87. Srivastava MD, Lippes J, Srivastava BI. Cytokines of the human reproductive tract. Am J Reprod Immunol 1996;36: 157-66.
88. Hunt JS, Chen HL, Hu XL, Pollard JW. Normal distribution of tumor necrosis factor-alpha messenger ribonucleic acid and protein in the uteri, placentas, and embryos of osteopetrotic (op/op) mice lacking colony-stimulating factor-1. Biol Reprod 1993;49:441-52.
89. Sharkey AM, Dellow K, Blayney M, Macnamee M, Charnock-Jones S, Smith SK. Stage-specific expression of cytokine and receptor messenger ribonucleic acids in human preimplantation embryos. Biol Reprod 1995;53:974-81.
90. Wijayagunawardane MP, Miyamoto A, Taquahashi Y, Gabler C, Acosta TJ, Nishimura M, et al. In vitro regulation of local secretion and contraction of the bovine oviduct: stimulation by luteinizing hormone, endothelin-1 and prostaglandins, and inhibition by oxytocin. J Endocrinol 2001;168:117-30.
91. Mazzali M, Kipari T, Ophascharoensuk V, Wesson JA, Johnson R, Hughes J. Osteopontin - a molecule for all seasons. QJM 2002;95:3-13.
92. Denhardt DT, Guo X. Osteopontin: a protein with diverse functions. FASEB J 1993;7:1475-82.

Received for publication: 3 August 2022. Accepted for publication: 27 February 2023.

European Journal of Histochemistry 2023; 67:3513
doi:10.4081/ejh.2023.3513