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Epithelial and neural cadherin expression in the mammalian reproductive tract and gametes and their participation in fertilization-related events

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ABSTRACT

Mammalian fertilization involves a series of well-orchestrated cell–cell interaction steps between gametes, as well as among spermatozoa and somatic cells of both the male and female reproductive tracts. Cadherins are Ca^{2+}-dependent glycoproteins that have been involved in cellular adhesion and signaling in somatic cells. Taking into account that Ca^{2+} ions are required during fertilization, the involvement of these proteins in adhesion events during this process can be anticipated. This report presents an overview on two members of classical cadherins, Epithelial (E-) and Neural (N-) cadherin in reproductive biology. Its provides evidence of studies done by several research groups about the expression of E- and N-cadherin during spermatogenesis, oogenesis and folliculogenesis, and their involvement in gamete transport in the reproductive tracts. Moreover, it describes current knowledge of E- and N-cadherin presence in cells of the *cumulus*-oocyte complex and spermatozoa from several mammalian species, and shows gathered evidence on their participation in different steps of the fertilization process. A brief summary on general information of both proteins is also presented.

Fertilization-related events: An overview

Mammalian fertilization involves a series of well-orchestrated steps of cell–cell interaction between spermatozoa and the oocyte as well as spermatozoa and somatic cells of the female reproductive tract while they transit to the fertilization site. The accomplishment of this process lies in the success of these interactions, which are based on complementary molecules present in the surface of both gametes. These events have been investigated at both the structural and molecular level, providing enormous insights toward establishing a "molecular pathway" for mammalian fertilization. The current view of this fascinating biological process involves the recognition and interaction of a group of proteins present in both gametes, resulting in generation of a viable zygote. This highly complex process begins with gamete production in the female and male gonads long time before gametes meet.

Oocytes arrested in meiotic prophase are stored in the ovary before birth as primordial follicles, surrounded by granulosa cells that support their growth after oogenesis and folliculogenesis. Following ovulation, the *cumulus*-oocyte complex transiently associates with the *fimbriae* oviductal cells; subsequently, the complex enters the *ampulla* portion of the oviduct, where they meet with spermatozoa to accomplish fertilization. On the other hand, spermatozoa complete their morphogenesis in the testis; the spermatogenesis is a multistep process that involves sequential mitosis and meiosis divisions by which the developing germ cell differentiates from spermatogonial stem cell to spermatogonia, spermatocyte, spermatid and then to spermatozoon. However, testicular spermatozoa are immature and must reside for some time in the epididymis, where they develop progressive motility and acquire ability to recognize and interact with the female gamete, in a process called epididymal sperm maturation. Spermatozoa that leave the epididymis mix with the secretions of the accessory glands at ejaculation and initiate a journey through the female reproductive tract. The male gametes are transported following guidance mechanisms as thermotaxis, rheotaxis and chemotaxis. During their transit, spermatozoa interact with the oviduct epithelial cells and undergo several structural and molecular modifications, collectively known as sperm capacitation, resulting in full acquisition of sperm fertilizing competence. Capacitated spermatozoa arrive to the *ampulla* to accomplish fertilization. They interact with the vestments that surround the oocyte in the *cumulus*-oocyte complex; sperm first interact with the *cumulus* cells and later with the oocyte extracellular matrix or *zona*

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0012-1606/

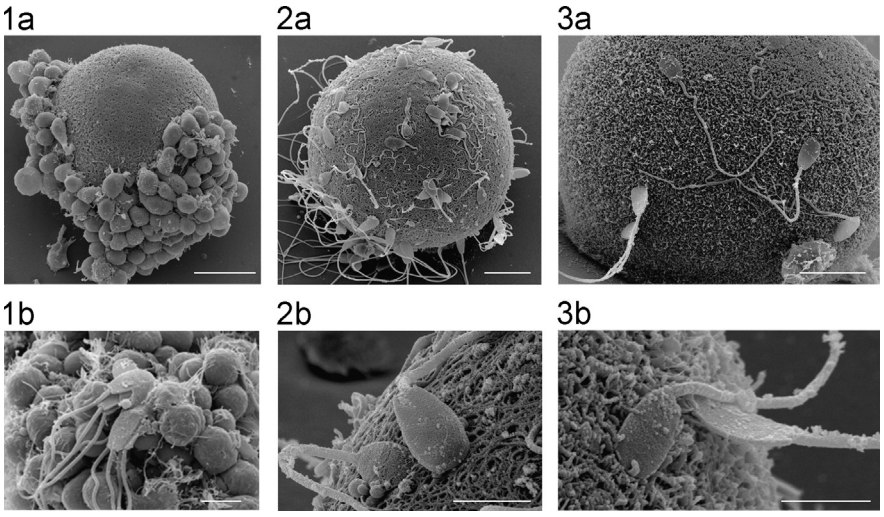

Fig. 1. Cell–cell adhesion events during bovine fertilization. Scanning electron microscopy images showing sperm–*cumulus* cells interaction (1a, detail in 1b), sperm–*zona pellucida* interaction (2a, detail in 2b) and sperm–oolemma interaction (3a, detail in 3b). Bar = 1a: 30 μm; 2a, 3a: 20 μm; 1b, 2b, 3b: 10 μm.

pellucida, to finally bind and fuse to the oocyte plasma membrane or oolemma. Before interacting with the oolemma, spermatozoa must undergo acrosomal exocytosis, a process wherein the sperm plasma and outer acrosomal membranes fuse and the acrosomal contents are released. Following sperm–oolemma fusion, sperm nucleus decondensation occurs, the male and female haploid pronucleae form, migrate towards each other and combine, generating the zygote. Fig. 1 shows a set of scanning microscopy images illustrating some of the cell–cell interaction events between sperm and vestments of *cumulus*-oocyte complex cells in the bovine model. The reader may access to publications from highly recognized experts in the field that summarize the molecular events mentioned above (some of them are listed in the following reviews: Yanagimachi, 1994; Wassarman, 1999; Primakoff and Myles, 2002; Talbot et al., 2003; Bedford, 2004; Eisenbach and Giojalas 2006; Vazquez-Levin and Marín-Briggiler, 2009; Visconti et al., 2011; Evans, 2012; Gadella, 2012; Okabe, 2013; Clift and Schuh, 2013; Klinovska et al., 2014).

Throughout these years, numerous efforts have been made toward the identification and functional characterization of the molecular entities involved in cell–cell adhesion events leading to the fertilization process. Regarding the identity of these proteins, some members of the immunoglobulin, integrin, and the selectin superfamilies have been found to be expressed in the gonads and gametes, and have been shown to participate in gamete production and interaction during fertilization. Table 1 presents some examples of cell–cell adhesion proteins found in spermatozoa and oocytes and, when found, evidence of their participation in fertilization-related events. The list includes some examples of members from the cadherin cell–cell adhesion molecules superfamily, a vast group of proteins that mediate calcium (Ca^{2+})-dependent adhesion and signaling.

Taking into account that gamete interaction requires the presence of Ca^{2+} ions (Fraser, 1987; Marín-Briggiler et al., 2003; Boni et al., 2007; Chen et al., 2013; Rahman et al., 2014) and that cadherins participate in Ca^{2+}-dependent cell–cell adhesion (Takeichi, 1995), the involvement of these proteins in adhesion events during fertilization could be anticipated. The present report summarizes a group of studies done by several research groups showing evidence on the expression of E- and N-cadherin during gametogenesis in both male and female gonads, and gamete transport in the male and female tracts, and their presence in cells from the *cumulus*-oocyte complex and spermatozoa, as well as evidence gathered on their participation in fertilization-related events. A brief overview on both proteins is first presented in the next section.

The cadherin superfamily: epithelial and neural cadherin

The cadherin superfamily is composed of a large group of cell surface (transmembrane or membrane-associated) glycoproteins. This superfamily is organized in at least five major families, among them type I or classical cadherins, type II closely related cadherins, desmosomal cadherins (desmocollins and desmogleins), protocadherins, and a variety of cadherin-related molecules. Members of this superfamily perform numerous functions that involved cell–cell recognition. Even though they have been mainly associated to cell–cell adhesion events, cadherins participate in numerous functions, among them cell–cell recognition, cytoskeletal organization, signal transduction and growth control (Takeichi, 1995; Gumbiner, 1996; Angst et al., 2001).

Epithelial cadherin (E-cadherin, Cadherin-1 (CDH1), L-CAM, ARC-1, uvomorulin) is the founder member of the cadherin superfamily (Takeichi, 1977). E-cadherin, a classical or type I cadherin, has been considered to be a paradigmatic classical cadherin and the prototype of all cadherin proteins because of its early identification and thorough characterization, both in normal and in pathological conditions. It was initially named uvomorulin based on early studies that described the ability of antibodies against the adhesion protein to block interactions between murine blastomeres, resulting in changes in embryo appearance that resemble a bunch of grapes (from latin "uva") (Hyafil et al., 1981; van Roy and Berx, 2008). Later studies demonstrated its role as a ubiquitous cell adhesion glycoprotein (Vestweber and Kemler, 1984). In the early 1980s, the name "cadherins" was introduced for this class of cell–cell adhesion molecules (Yoshida-Noro et al., 1984). E-cadherin is an essential glycoprotein for development, cell differentiation and tissue homeostasis, as well as for maintenance of epithelial polarity and structural integrity (van Roy and Berx, 2008).

The *CDH1* gene encodes human E-cadherin, is located on chromosome 16q22.1, and spans a region of approximately 100 kb; it comprises 16 exons and 15 introns and is highly conserved among species (Berx et al., 1995). Cloning of mouse E-cadherin led to the prediction of its structure composed of a signal sequence, a propeptide and a one-pass-transmembrane glycopolypeptide (Nagafuchi et al., 1987) and cloning of the human

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Table 1
Examples of **cell–cell** adhesion proteins found in spermatozoa and oocytes and evidence of their participation in fertilization-related events.

Adhesion molecules	Gamete expression	Involvement in fertilization-related events	References
Immunoglobulins[a]			
Basigin	S and O	Sperm–*zona pellucida* interaction	Ding et al. (2002), Saxena et al. (2002), Saxena and Toshimori (2004)
CD4	S and O	Homologous fertilization	Gobert et al. (1990), Mori et al. (1991), Guo et al. (1995)
ICAM-1, NCAM, VCAM-1	O	Embryo development	Kimber et al. (1994), Campbell et al. (1995)
EWI-2	O	Sperm–oolemma interaction	Glazar and Evans (2009)
Growth factor receptors	S and O	Sperm capacitation, acrosomal exocytosis Oocyte maturation	Yoshida et al. (1998), Einspanier et al. (2002), Prochazka et al. (2003), Cotton et al. (2006), Breitbart and Etkovitz (2011)
Izumo	S	Sperm–oolemma fusion	Inoue et al. (2005), Hayasaka et al. (2007), Kim et al. (2013)
JAM-A	S	Sperm motility	Shao et al. (2008)
MHC class II	S	Homologous fertilization	Mori et al. (1990)
Selectins			
L-selectin	S and O	Sperm–*zona pellucida* interaction	Campbell et al. (1995), Lucas et al. (1995)
P-selectin	S	Sperm–*zona pellucida* interaction Sperm–oolemma interaction	Fusi et al. (1996a), Geng et al. (1997)
Integrins			
Different α and β subunits	S and O	Sperm–oolemma interaction Oocyte activation	Tarone et al. (1993), Fusi et al. (1993, 1996b), Almeida et al. (1995), Campbell et al. (1995), Takahashi et al. (2000), Tatone and Carbone (2006), Barraud-Lange et al. (2007), Vjugina et al. (2009)
Cadherins			
E-cadherin	S and O	Sperm–oviduct interaction Sperm–*zona pellucida* interaction Sperm–oolemma interaction Homologous fertilization	Campbell et al. (1995), Rufas et al. (2000), Ziv et al. (2002), Purohit et al. (2004), Marín-Briggiler et al. (2008), Takezawa et al. (2011), Caballero et al. (2014)
N-cadherin	S and O	Sperm–oolemma interaction	Goodwin et al. (2000), Rufas et al. (2000), Ziv et al. (2002), Marín-Briggiler et al. (2010)
P-cadherin	S and O	–	Rufas et al. (2000), Ziv et al. (2002)
Others	S	–	Johnson et al. (2004)

S=spermatozoa; O=oocyte.

[a] This superfamily comprises more than 700 known proteins, examples of some members of this superfamily are provided.

homolog confirmed high conservation among species and other members of the family (Bussemakers et al., 1993). The mature protein is a ~120 kDa glycoprotein organized in an ectodomain composed of five extracellular domains of around 110 amino acids each (Blaschuk and Rowlands, 2002), a single-pass transmembrane domain and a highly conserved carboxy-terminal cytoplasmic domain of approximately 150 amino acids, a characteristic structure of all members of this family (Table 2). E-cadherin extracellular domains mediate mainly homophilic cell–cell adhesion between adjacent cells (Blaschuk et al., 1990; Nose et al., 1990; Ozawa and Kemler, 1998). The first extracellular domain contains the HAV amino acid sequence necessary for cadherin–cadherin binding, and the amino acids adjacent to the tripeptide sequence are necessary for the recognition specificity of cadherin proteins. The Ca^{2+} ions are essential for cadherin-mediated cell adhesion and bind to specific sequences present on each domain of the ectodomain. Also, specific sequences of the E-cadherin intracellular domain interact with several proteins (among them α-, β-, δ1-/p120, δ2- and γ-/plakoglobin catenins (official symbols CTNNA1, CTNNB1, CTNND1, CTNND2 and CTNNG, respectively), that form a complex link to the actin cytoskeleton, which regulates the strength of the cadherin-mediated cell adhesion, and/or are involved in signal transduction pathways (Nagafuchi et al., 1993; van Roy and Berx, 2008; Paredes et al., 2012). Interactions of E-cadherin with catenins and Ca^{2+} appear to be essential for its adhesive properties (Chitaev and Troyanovsky, 1998).

Among adaptor proteins, β-catenin is a central component of the cadherin/catenin adhesive complex, since binding to cadherin remains a prerequisite for adhesion due to its role in protecting the cadherin cytoplasmic domain from rapid degradation as well as enhancing the efficiency of endoplasmic reticulum to cell surface transport and recruiting α-catenin to sites of cell–cell contact. Post-translational modifications (i.e. phosphorylation by Src and casein kinases) that regulate the interaction between β-catenin and cadherin have relevant consequences for cell–cell adhesion (Daugherty and Gottardi, 2007).

Under physiological conditions, localization of E-cadherin is restricted to cell–cell contact sites; part of the cell surface E-cadherin is subjected to endocytosis and recycling, providing a mechanism to modulate E-cadherin surface expression and cell adhesion (Bryant and Stow, 2004; Mosesson et al., 2008). In normal tissues, E-cadherin is mainly expressed in epithelial cells and plays an important role in the formation and maintenance of normal tissue architecture and function. Several mechanisms have been associated to modulate E-cadherin expression and function (s), among them post-translational modifications (i.e. proteinase processing, phosphorylation and glycosylation) as well as transcriptional repression, promotor methylation, somatic and hereditary mutations and loss of heterozygosity (van Roy and Berx, 2008). E-cadherin has been defined as a growth and invasion tumor suppressor and has been frequently found downregulated in epithelial tumors, a process that leads to cell motility, invasion and metastasis. This process, called “epithelial to mesenchymal transition”, recapitulates molecular and functional changes identified during embryogenesis (Lim and Thiery, 2012).

Using gene knock-out technologies, two studies demonstrated defective preimplantation development due to embryonic lethality in mice with a targeted null mutation in the E-cadherin gene (Larue et al., 1994; Riethmacher et al., 1995). Whereas heterozygous mutant animals appeared normal and fertile, viable homozygous mutant animals were absent in the offspring from matings of heterozygous mice. E-cadherin-null embryos were found to develop into abnormal blastocysts which could not implant in the uterus; they were initially able to compact mainly due to the presence of residual maternal E-cadherin previously reported (Sefton et al., 1992), but the adhesive properties of the morula cells were

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Table 2
CDH1 and CDH2 gene attributes.

Term	*CDH1*	*CDH2*
Gene name (synonyms)	CDH1, CDHE, UVO	CDH2, CDHN, NCAD
Chromosome localization	16 (16q22.1)	18 (18q11.2)
Gene structure	16 exons, 15 introns	16 exons, 15 introns
Protein name	Cadherin-1 (E-cadherin)	Cadherin-2 (CDw245; Neural Cadherin; CD_antigen: CD325) (N-cadherin)
Uniprot accession	P12830	P19022
Protein precursor	882 aa	906 aa
Mature protein (Mr)	728 aa (120 kDa)	747 aa (135 kDa)
Protein structure	Signal peptide: 1–21 (21 aa) Propeptide: 23–154 (132 aa) EC domain (EC1–EC5): 155–709 (550 aa) EC1:155–262 (108 aa); EC2: 263–375 (113 aa); 376–486 (111 aa); EC4: 487–593 (107 aa); EC5: 594–697 (104 aa) Transmembrane Domain: 710–730 (21 aa) Cytoplasmic Domain: 731–882 (152 aa)	Signal peptide: 1–25 (25 aa) Propeptide: 26–159 (134 aa) EC domain (EC1–EC5): 160–724 (565 aa) EC1:160–267 (108 aa); EC2: 268–382 (115 aa); 383–497 (115 aa); EC4: 498–603 (106 aa); EC5: 604–714 (111 aa) Transmembrane Domain: 725–745 (21 aa) Cytoplasmic Domain: 747–906 (161 aa)
Reactome (GO biological process)	Adherens junction organization Apoptotic process Cell junction assembly Cell–cell junction organization Cellular component disassembly involved in execution phase of apoptosis	Adherens junction organization Blood vessel morphogenesis Ca^{2+}-dependent cell–cell adhesion Cell adhesion Cell junction assembly Cell migration Cell–cell junction organization Heterophilic cell–cell adhesion Homophilic cell adhesion Muscle cell differentiation Negative regulation of canonical Wnt Signaling pathway Positive regulation of MAPK cascade Positive regulation of muscle cell differentiation Striated muscle cell differentiation

aa: amino acid.
Information extracted from the SIB ExPASy Bioinformatics Resource Portal (http://www.expasy.org/). Artimo et al. (2012).

significantly reduced, resulting in blastomere dissociation shortly after compaction occurred. Interestingly, anti-E-cadherin blocking antibodies inhibited initial embryonic compaction of E-cadherin-null embryos, suggesting that maternal E-cadherin function would not be compensated by other molecules. The studies also revealed that E-cadherin is essential for cell polarity in the early mouse embryo, since morphological polarization was severely affected in the mutant embryos after the morula stage.

N-cadherin (official symbol CDH2) is another classical cadherin originally identified as a cell–cell adhesion molecule expressed in neural cells, and later found in other cells and tissues, among them cardiac muscle, kidney, stomach, liver, thymus, pituitary, pancreas and adrenal cells (Volk and Geiger, 1984; Hatta et al., 1985; Nouwen et al., 1993; Nuruki et al., 1998; Tsuchiya et al., 2006). Human N-cadherin is located on chromosome 18q11.2, has no linkage to the other classical cadherins, and is well separated from the desmosomal cadherin locus and from another minicluster in the same chromosome containing other cadherin genes (Angst et al., 2001). The N-cadherin gene is organized in 16 exons and 15 introns, and is transcribed to a unique messenger (m) RNA that encodes a 135 kDa mature protein (Table 2). N-cadherin has also been involved in embryonic development, implantation, tissue formation and maintenance of tissue integrity during adulthood. At synaptic contacts, N-cadherin is commonly localized adjacent to the active zone and the post-synaptic density, suggesting that N-cadherin contributes to the assembly of the synaptic complex. Moreover, in tumors in which E-cadherin expression is reduced, N-cadherin is commonly upregulated, a process known as the cadherin 'switch'. This process is considered hallmark of the epithelial to mesenchymal transition. N-cadherin expression has been related with increased tumor cell migration, invasion and metastasis, and with tumor angiogenesis and maintenance of tumor vasculature (Nakagawa and Takeichi, 1998; Suyama et al., 2002; Marambaud et al., 2003; Derycke and Bracke, 2004; van Roy, 2014).

Despite the striking similarities with E-cadherin, N-cadherin has specific structural features and several distinctive functions (Table 2). As indicated above, identical E-cadherin molecules preferentially bind in *trans* across cell–cell contacts on cell plasma membranes in homophilic binding. N-cadherin mediates homotypic binding between apposed cell membranes and regulates the actin cytoskeleton through protein interactions with the cytoplasmic domain. However, during tumor progression, the induction of N-cadherin expression in some cells of epithelial origin enables them to interact with other cell types (heterotypic cell–cell adhesion), such as stromal cells and endothelial cells, which normally express N-cadherin. Moreover, N-cadherin promotes nuclear β-catenin activity. Mechanistically, N-cadherin is believed to functionally interact with the fibroblast growth factor receptors (FGFR), and cause a sustained FGFR signaling and progression of carcinoma; E-cadherin does not interact with the FGFR.

Null mice for N-cadherin were also generated by gene knock-out technologies (Radice et al., 1997). Similarly to the E-cadherin-null model, heterozygous mice were of normal size and fecundity when compared with their wild-type littermates, and no mice homozygous for the N-cadherin were detected among all progeny analyzed. Embryos homozygous for the N-cadherin mutation depicted a severe cell adhesion defect in the heart, and cardiac myocytes failed to generate a normal myocardium; embryos died by day 10 of gestation. Despite the fact that neurulation and somitogenesis were demonstrated to occur in the N-cadherin-null embryos, the resulting structures were reported to be abnormal. Altogether, these studies

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demonstrated the relevance of N-cadherin for proper myocardium and yolk sac development in the mouse.

E- and N-cadherin in reproductive tissues and gametes

Maintenance of appropriate cell–cell contact is a key element in the physiology of reproductive tissues. It is, therefore, understandable that evidence on the expression of several adhesion proteins, among them E- and N-cadherin, has been described in some normal human reproductive tissues, including ovary, oviduct, uterus, mammary gland, testis, epididymis and prostate (i.e. Inoue et al., 1992; Andersson et al., 1994; Delmas and Larue, 2004; Tsuchiya et al., 2006). These proteins exert very relevant functions, acting as regulators of organogenesis as well as in tissue function. In the following sections, an overview on the expression and function of E- and N-cadherin during gametogenesis and gamete transport, as well as in both *cumulus*-oocyte complexes and spermatozoa and their participation in fertilization-related events will be presented. Most of the reports found have addressed the expression of cadherin transcripts and proteins in tissues and cells, but there is yet scarce information on their function and the underlying mechanisms/pathways involved.

E- and N-cadherin in gametogenesis and sperm transport to the fertilization site

Early patterning of the mammalian gonad

The molecular basis responsible for early patterning of the mammalian gonad developed from a bipotential state into a testis or an ovary have yet not been unraveled. Whereas sex-specific vascularization has been found essential in this process, other mechanisms required to establish either cord or interstitial compartments in the testis as well as ovarian cords are yet unknown. Specifically regarding adherent junctions in sex-specific patterning of the gonad, the relevance of E-cadherin in the developing mouse was evaluated (Mackay et al., 1999; Okamura et al., 2003). While germ cells in the indifferentiated gonad showed strong immunoreactivity towards the adhesion protein, somatic cells were only stained when depicted cell contact with germ cells. Positive staining for E-cadherin was found in epithelial cells of the mesonephric duct and tubules and at later stages, germ cell immunoreactivity was correlated with stages of ovarian differentiation, being reduced or absent between germ cells at 16 days post *coitum* (prelude to follicle formation). Stronger staining reappeared only briefly at 17 days post *coitum*, the time of follicular cell attachment to oocytes. In the differentiating testis, immunoreactivity was initially restricted to the germ cell population but pre-Sertoli cells were strongly positive between 16 and 19 days post *coitum*. The most striking sex difference was seen in somatic cell population, with Leydig cells becoming strongly positive for E-cadherin from 17 days post *coitum* onwards. In support of E-cadherin involvement in cell–cell interactions on developing gonad, dissociated cells from gonads of either sex were unable to reform their initial contacts when cultured in the presence of the anti E-cadherin antibody.

In a recent study, both membrane-associated β-catenin and p120-catenin were found to co-localize with cell-specific cadherins in both sex-nonspecific and sex-specific patterns at the time of early gonadal sex differentiation (Fleming et al., 2012). This report also showed a relationship between protein expression patterns and influence of adherent junctions in the overall patterning of the testis versus the ovary, following known cell–cell adhesion mechanisms of the adherent junctions. Thus, suggesting that cadherin-mediated junctions are essential effectors of patterning within the developing male and female gonads.

Spermatogenesis

As indicated above, spermatogenesis is a tightly regulated developmental process that involves sequential mitotic and meiotic cellular divisions, by which developing germ cells differentiate from spermatogonia to spermatocytes, spermatids and finally to spermatozoa. To complete spermatogenesis, differentiating germ cells must migrate through the seminiferous epithelium while maintaining transiently attached to Sertoli cells. This process is associated with temporal and spatial expression of adhesion molecules and with the formation of specific junctions between neighboring Sertoli cells, and between Sertoli and germ cells. In the seminiferous epithelium, the major co-existing and co-functioning junction types described are anchoring or adherent junctions, gap junctions and occluding or tight junctions (Byers et al., 1993; Kerr et al., 2006).

Anchoring junctions interconnect cytoskeletal proteins of adjacent cells and three morphologically distinct adherent junctions have been found in the mammalian testis: desmosome-like junctions, ectoplasmic specializations and tubulobulbar complexes (Kopera et al., 2010; Upadhyay et al., 2012). Desmosome-like junctions are intermediate filament-based junctions located between Sertoli cells and germ cells (except elongated spermatids) (Lie et al., 2011). When round spermatids differentiate into elongated spermatids, the adhesive function of desmosome-like junctions is replaced by ectoplasmic specializations, which are testis-specific adherent junctions, composed of actin filaments, endoplasmic reticulum, and microtubules subjacent to the Sertoli cell plasma membrane. The ectoplasmic specializations between elongated spermatids and Sertoli cells are known as apical ectoplasmic specializations, but ectoplasmic specializations are also found in the basal portion of the Sertoli cells, at regions of neighboring Sertoli cell attachment (basal ectoplasmic specializations) (Lee and Cheng, 2004; Wong et al., 2008). Tubulobulbar complexes are testis-specific actin-related endocytic structures found both at adhesion junctions between Sertoli–Sertoli cells (basal tubulobulbar complexes) and between Sertoli cells and elongated spermatids (apical tubulobulbar complexes). Apical tubulobulbar complexes form where ectoplasmic specializations begin to disassemble, and play a relevant function during sperm release or spermiation and in junction remodeling (Vogl et al., 2014). Gap junctions are involved in cellular communication in the seminiferous epithelia and are comprised by aggregated channels that allow the exchange of small regulatory proteins and ions between cells (Pointis et al., 2010). Tight junctions exist between adjacent Sertoli cells and have an essential role in testis compartimentalization. These junctions, together with basal ectoplasmic specializations, desmosome-like junctions, basal tubulobulbar complexes and gap junctions, form the blood–testis barrier that divides the seminiferous tubule into a basal and an adluminal compartment, creating an immunologically protected microenvironment in which germ cells can differentiate. Spermatogenesis involves germ cell migration from the basal to the adluminal compartment, with a dynamic assembly/disassembly of the blood–testis barrier (Lui et al., 2003; Wong and Cheng, 2005; Mruk and Cheng, 2010).

Although the morphology of cellular junctions in the mammalian testis has been widely described, their molecular composition and regulatory mechanisms are still poorly understood. The vast literature on cellular adhesion during spermatogenesis has been compiled in several excellent reviews (Cheng and Mruk, 2002, 2009; Lui and Cheng, 2012).

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In the last 20 years, numerous reports have evaluated the expression of cadherin proteins in the seminiferous epithelium and assessed their role in cell–cell adhesion events during spermatogenesis. However, there is much controversy regarding localization and functions of cadherin and related proteins in the mammalian testis, in particular their involvement in the formation of the testis-specific adherent junctions, the ectoplasmic specializations (Goossens and van Roy, 2005). Cyr et al. (1992a) first described the expression of E-, N- as well as Placental (P)-cadherin transcripts in the rat testis and showed dramatic changes of each cadherin expression associated with the development of the fetal gonad into the mature testis. Further studies reported the expression of more than 24 classic cadherin and protocadherin mRNAs in the fetal, immature and adult mouse and rat gonads. Most of these cadherins were found to be expressed in the fetal gonad, but their levels (with the exception of N-cadherin) were decreased after birth, emphasizing the relevance of these adhesion molecules in testicular development (Munro and Blaschuk, 1996; Johnson et al., 2000). Regarding E-cadherin, its expression was reported to be maximal in the fetal testis, and was still detected in the immature gonad before the formation of the blood–testis barrier (Wu et al., 1993; Munro and Blaschuk, 1996; Johnson et al., 2000). However, there is no consensus about E-cadherin expression in the adult testis; while some studies did not detect either E-cadherin transcript or protein in the mammalian adult tissue (Cyr et al., 1992a; Andersson et al., 1994; Munro and Blaschuk, 1996; Tsuchiya et al., 2006), recent findings indicate its expression in undifferentiated spermatogonia (Tokuda et al., 2007; Zhang et al., 2011).

Several lines of evidence show N-cadherin expression in the seminiferous epithelium, with maximal transcript levels in the 42-day-old rat testis (Cyr et al., 1992a) and in the 21-day-old and adult mouse gonad (Munro and Blaschuk, 1996), suggesting a role of N-cadherin in spermatogenesis. Studies performed in the rat model have shown that N-cadherin is expressed by isolated Sertoli and germ cells (Chung et al., 1998), and that this protein is present within the basal compartment of the seminiferous epithelium at inter-Sertoli and Sertoli–germ cell junctions, but also within the adluminal compartment at the heads of elongating spermatids, in a stage-specific manner (Johnson and Boekelheide, 2002). In the adluminal compartment, N-cadherin was described to be associated with intracellular proteins such as β-catenin, p120, desmoglein, Src and the carboxyl-terminal Src kinase (Csk) and was involved in the spermiation process (Wine and Chapin, 1999). Functional studies have shown the participation of N-cadherin in rat Sertoli cell–germ cell adhesion, since specific antibodies partially inhibited these cell–cell interactions (Newton et al., 1993). Such interactions seem to be hormonally regulated, as the production of N-cadherin by Sertoli cells and the binding of round spermatids to Sertoli cells *in vitro* were synergistically stimulated in the presence of steroids (testosterone or estradiol) and follicle-stimulating hormone (FSH) (Perryman et al., 1996; MacCalman et al., 1997). Our studies demonstrated a N-cadherin transcript expression in the human testis almost 100 times higher than that of the epididymis and reported the presence of the 135 kDa N-cadherin protein in whole human testicular protein extracts (Marín-Briggiler et al., 2010), in agreement with other reports describing its presence in spermatogonia, primary spermatocytes and Sertoli cells (human: Andersson et al., 1994; Tsuchiya et al., 2006; mouse: Veiga/Marín-Briggiler et al., unpublished).

There is much controversy in the literature regarding the involvement of the cadherin/catenin complex as the functional unit of ectoplasmic specializations in the seminiferous epithelium, and the precise cytoskeletal attachment site of this complex. It has been reported that N-cadherin did not localize at basal ectoplasmic specializations (Johnson and Boekelheide, 2002), but it would be expressed at the desmosome-like junctions of Sertoli cells, associated with p120 catenin, β-catenin and the intermediate filament-based cytoskeleton (Mulholland et al., 2001; Johnson and Boekelheide, 2002). In contrast, other reports have demonstrated the existence of a N-cadherin/β-catenin complex, linked to the actin cytoskeleton, between Sertoli and germ cells near the basal and the lower one-third of the adluminal compartment (Lee et al., 2003). Moreover, the presence of N-cadherin but the absence of studied catenins at the adluminal compartment has led to propose that N-cadherin would serve a signaling rather than a structural function in this compartment (Byers et al., 1994; Mulholland et al., 2001). At least some of these conflicting results appear to be attributed to differences in the antibody specificities used in different studies.

Further studies have identified the adaptors zyxin, axin and Wiskott–Aldrich syndrome protein (WASP) (Lee et al., 2004), and several kinases and phosphatases (as Fer kinase, the myotubularin-related protein 2 (MTMR2)/c-Src protein complex, c-Yes (Chen et al., 2003; Lee and Cheng, 2005; Zhang et al., 2005; Xiao et al., 2011)), which are associated to N-cadherin/β-catenin complexes in the testis and might modulate Sertoli–germ cell adherent junction dynamics. Additionally, there is evidence that the N-cadherin/β-catenin-mediated adhesion function in the testis is regulated by the nitric oxide synthase/cGMP/protein kinase G/β-catenin signaling pathway (Lee et al., 2005) and by soluble guanylate cyclase β1 (Sarkar et al., 2006). Current efforts are focused in gaining knowledge on the mechanisms that allows adherent junction disruption and N-cadherin recycling in the seminiferous epithelium that facilitates germ cell movement without compromising the blood–testis barrier integrity (Yan et al., 2008; Su et al., 2010).

In a recent report, a lack of desmosome-like junctions in the mammalian seminiferous epithelium, but rather the presence of adherent junctions formed by N-cadherin anchored in cytoplasmic plaques was proposed; in addition, authors described the presence of novel type of junction structures between Sertoli cells (Domke et al., 2014). Further research will help to elucidate this topic.

Epididymal sperm maturation

Spermatozoa released from the testes undergo changes in several cell subdomains during epididymal transit. These modifications refer to a process called epididymal sperm maturation, an essential post-testicular event in the acquisition of fertility by the male gamete. The epididymis, a highly convoluted duct system, has been organized into three anatomical regions: *caput*, *corpus* and *cauda*. In rodents, a proximal segment distinct from the *caput* region is named the initial segment, and depicts unique histological characteristics. In addition to concentrate spermatozoa in the proximal segments and to store and protect the male gamete prior to ejaculation, the epididymal epithelium provides a defined sperm environment to accomplish maturation (Robaire and Hermo, 1988; Turner, 1995; Cornwall, 2009; Dacheux and Dacheux, 2013).

An adequate epididymal luminal *milieu* is possible due to the blood–epididymal barrier, together with the endocytic and secretory functions of the epithelial cells (Cyr et al., 2007). Similarly to the blood–testis barrier, the blood–epididymal barrier is a complex anatomical, physiological and immunological restrictive barrier, which is critical for protecting spermatozoa and for regulating the luminal environment composition of the epididymis, achieved by a differential transport of specific molecules across the cells by means of solute protein carriers, channels and receptors. The blood–epididymal barrier is composed of both adhering junctions that are necessary for cell adhesion and intercellular signaling, and tight junctions, which form the seal between adjacent epithelial cells (Cyr et al., 2002; Turner, 2002). Gene expression profile analysis has identified a significant number of genes involved in cell–cell adhesion processes, among them cadherins, catenins and

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claudins, to be highly expressed in the *caput* human epididymis (Dubé et al., 2007; Thimon et al., 2007), stressing the relevance of cell–cell molecules in the maintenance and function of this reproductive organ. In particular, E-cadherin has been related to the establishment and function of the blood–epididymal barrier. A detailed analysis on the expression of E-cadherin in the epididymis was first reported in the rat in the early 1990s (Cyr and Robaire, 1991; Cyr et al., 1992b). These investigations demonstrated E-cadherin mRNA expression in the entire epididymis, showing a differential distribution along this organ, and a regulation by circulating androgens. Specifically, higher E-cadherin mRNA expression levels were reported in the *caput* and *corpus* than in the initial segment and in the *cauda* regions by means of mRNA of Northern blot analysis. In the same study, the E-cadherin polypeptide was immunodetected only in the principal cells of all regions, depicting the highest signal in the *corpus* (Cyr et al., 1992b). Using electron microscopy, E-cadherin was immunolocalized in the epididymal principal cells, between the lateral plasma membranes of adjacent principal cells, both at the level of apically located junctional complexes and in the deeper underlying areas (Cyr et al., 1995). Other studies from the same group and others addressed the post-natal developmental changes in E-cadherin mRNA expression and protein localization in the rat epididymis, demonstrating a segment-specific and age-dependent regulation of E-cadherin (Cyr et al., 1992b; Levy and Robaire, 1999; Johnston et al., 2005). In agreement with these findings, a recent study reported the effect of miR-200c expression (lowest at Day 7, highest at Day 36 and followed by a dramatic decrease) upon E-cadherin mRNA levels, by regulating the TCF8/ZEB1 transcription factor mRNA during post-natal epididymal development in juvenile rats (Wang and Ruan, 2010). Other studies reported the expression of classical cadherins in the epididymis during mouse embryonic development, on tissue sections of 14.5, 16.5, 18.5 and P1-days male embryos using fluorescent immunohistochemistry with a pan-cadherin antibody (Snyder et al., 2010). In the early studies, the androgen-dependence of E-cadherin mRNA expression was evidenced by the significant decrease observed after bilateral orchidectomy and its restoration after testosterone supplementation (Cyr et al., 1992b). More recently, using a microarray mRNA expression analysis, N- and P-classical cadherin were also detected in the human epididymis, although at lower levels (Cyr et al., 2007). In a study done by our group, quantitative Real Time PCR analysis resulted in the detection of neglible N-cadherin expression levels in the whole epididymis compared to those found in the testis (Marín-Briggiler et al., 2010).

With regard to the cadherin adaptor proteins, studies thoroughly demonstrated the expression of α-, β- and δ1/p120- catenins in the adult rat epididymis. Whereas α-catenin and β-catenin expression was maximal in the *corpus* and *cauda* epididymis and dependent of androgens, δ1/p120 expression was intense and similar in all epididymal regions and was not altered after bilateral orchidectomy (DeBellefeuille et al., 2003). In line with the findings described above, a recent study done in adult pig epididymis reported down-regulation of E-cadherin and β-catenin transcripts in early post-natal and prepubertal animals exposed to the anti-androgen flutamide injected on post-natal-day 2 (Gorowska et al., 2014). Protein immunolocalization studies revealed a specific signal for the three catenins along the lateral plasma membrane between adjacent epithelial cells, suggesting their participation in the adherent complex. In agreement with the immunolocalization analyses, immunoprecipitation studies demonstrated the interaction of E-cadherin with the catenin adaptor proteins (DeBellefeuille et al., 2003). These studies were highly relevant, taking into account that formation of tight junctions was proposed to first involve the formation of cadherin-based cell adhesion, followed by the recruitment of the tight junctional cytoplasmic protein zona occludens-1 (ZO-1) to the lateral cell plasma membrane by direct interaction with catenins.

Our group reported the expression of the E-cadherin mRNA in the three tissue segments of the human epididymis (Marín-Briggiler et al., 2008). A quantitative mRNA analysis revealed a higher expression of the transcript encoding the adhesion protein in the *caput* and *corpus* regions than in the *cauda* segment, in agreement with a study that reported the distal epididymal section having the lowest transcriptional activity (Thimon et al., 2007), but differing from another study that described similar expression levels in all three segments (Dubé et al., 2007). Regarding the protein, Western immunoblotting results from our study showed presence of the 120 kDa mature E-cadherin protein in total epididymal extracts; by immunohistochemical analysis, the adhesion protein was immunodetected in the principal epithelial cells from the three segments of the human epididymis (Marín-Briggiler et al., 2008), in agreement with a previous report (Andersson et al., 1994). Similar results were obtained in epididymal tissue sections from mouse and bull epididymis (Caballero/Veiga et al., unpublished), leading us to suggest the involvement of E-cadherin in the formation and maintenance of the blood–epididymal barrier, as previously suggested in rodent models (Levy and Robaire, 1999). In *cauda* epididymal plasma and in the soluble fraction of the seminal plasma, the 86 kDa E-cadherin ectodomain was detected (Marín-Briggiler et al., 2008), that would result from the "shedding" mediated by Matrilysin (MAT, MMP-7, EC 3.4.24.23), an enzyme expressed in many tissues, among them in the mouse epididymis (Wilson et al., 1995).

In addition to its role in the blood–epididymal barrier, E-cadherin may have an active participation in the sperm maturation process and acquisition of maturating epididymal proteins. It is well accepted that sperm maturation requires the interaction of spermatozoa with proteins that are synthesized and secreted in a region-specific manner by principal cells of the epididymal epithelium (Robitaille et al., 1991; Gatti et al., 2004). The molecular mechanisms underlying sperm maturation are yet unresolved, but there is evidence suggesting the role of extracellular vesicles released by apocrine secretion from the epididymal epithelium, named epididymosomes, in transfer to the spermatozoon of proteins proposed to be involved in sperm–egg interaction and motility and other functions (Sullivan and Saez, 2013). After immunostaining of tissue sections, E-cadherin protein was localized to the apical surface of epididymal epithelial cells; moreover, additional evidence has been collected suggesting its detection in epididymosome-like membranous structures present in the murine *cauda* epididymal plasma (Veiga et al., unpublished) and reported in prostasome-like particles present in human seminal plasma (Marín-Briggiler et al., 2008).

Oogenesis and folliculogenesis

Oogenesis and folliculogenesis are complex and coordinated biological processes occurring in the ovary. They involve a series of events that induce morphological and functional changes within the follicle, leading to cell differentiation and oocyte development. During fetal life, oogonia proliferate through mitosis to populate the ovarian cortex. Subsequently, they enter meiosis I and these oocytes are arrested at diplotene of prophase I until they are fully-grown. At this stage, the oocyte, called germinal vesicle, is located within a primordial follicle characterized by being surrounded by a basement membrane, and a single layer of flat granulosa cells. Shortly after birth and throughout life, folliculogenesis occurs, a process by which oocyte meiotic and developmental competence is gradually gained. These events comprise an extensive remodeling of primordial follicles, giving rise to antral follicles containing the oocyte surrounded by granulosa cells, also called *cumulus*

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oophorus. During ovulation, the mature oocyte is released to the peritoneal cavity and the remaining follicular cells differentiate into the *corpus luteum* in a process known as luteinization (Greenwald and Roy, 1994; Espey and Lipner, 1994; Hirshfield, 1991; McGee and Hsueh, 2000; Edson et al., 2009; Sánchez and Smitz, 2012).

Cell–cell interactions are an important aspect of ovarian function and structure. It is known that adhesion molecules play an important role in ovarian tissue development and remodeling processes during folliculogenesis as well as after ovulation and *corpus luteum* formation (Machell and Farookhi, 2003). In addition to E-cadherin involvement in the establishment of the germ cell lineage (see above), it participates in oocyte growth, and in the acquisition of meiotic competence during gonad development in mice (Carabatsos et al., 2000). On the other side, N-cadherin-mediated adhesion of granulosa cells in culture prevents ovarian cells from dying via apoptosis (Peluso et al., 1996; Trolice et al., 1997; Makrigiannakis et al., 1999), suggesting that both adhesion proteins play a highly relevant role in folliculogenesis.

The expression of E- and N-cadherin proteins in the ovary has been examined in several species, depicting a differential spatio-temporal expression fashion during mammalian folliculogenesis. Early studies done in the pig detected presence of E-cadherin protein in the ovary, being highest in fetal and neonatal tissues and declining at 16 weeks to adulthood, and a similar trend was observed for the transcript (Ryan et al., 1996). In the rat ovary, N- and E-cadherin mRNA (RT-PCR) and protein (Western immunoblotting) expression evaluated from 19–20 days of gestation to 25 days post-partum was detected at all the ages examined, showing a peak for both proteins at 7 days of age (Machell et al., 2000). E-cadherin expression was reported to remain exclusively in the oocyte during neonatal hamster ovary development, while N-cadherin expression was found to shift from oocytes to granulosa cells of primordial follicles on post-natal day 8, and a strong N-cadherin expression was restricted to granulosa cells of growing follicles subsequently. In this study, ovarian E- and N-cadherin mRNA levels were found to decrease from embryonic day 13 through post-natal day 3 and 10, respectively, and protein accompanied the mRNA expression (Wang and Roy, 2010). A thorough study done in the mouse (Mora et al., 2012) reported the expression of E- and N-cadherin transcripts in total ovaries from animals between 4 days and 3 weeks of age by means of RT-PCR.

In the human model, a study done on a group of 8–20 weeks' gestation fetal ovaries showed E- and N-cadherin expression at all gestations, with overlapping although distinctive patterns. E- and N-cadherin were immunodetected in germ cells and adjacent somatic cells, but no expression was detected in the somatic cell cords. The epithelia of the Wolffian and Müllerian ducts expressed only E- and N-cadherin, respectively, in a mutually exclusive fashion. This pattern of cadherin expression was found to be conserved between human and rat fetuses of both genders (Smith et al., 2010). N-cadherin was also reported to be expressed in the human adult ovary (Tsuchiya et al., 2006) and disruption of N-cadherin mediated cell–cell adhesion was associated with apoptosis of human granulosa cells as well as ovarian surface epithelial cells (Makrigiannakis et al., 1999; Pon et al., 2005).

E- and N-cadherin expression in the ovary has been found to be regulated by several hormones (MacCalman et al., 1994, 1995; Farookhi et al., 1997; Sundfeldt et al., 2000; Wang and Roy, 2010). In the ovary of fetal hamster, neutralization of FSH in utero was reported to impair primordial follicle formation with an associated decrease in N-cadherin mRNA and protein, but an increase in E-cadherin transcript and protein. This altered expression was reversed by equine chorionic gonadotropin (eCG) treatment (Wang and Roy, 2010). In the rat, E-cadherin expression in theca and interstitial cells of immature ovaries was found before and after injection of eCG, and decreased after an ovulatory dose of human chorionic gonadotropin (hCG). In the same report, authors described a restricted expression of E-cadherin in granulosa cells of preantral follicles, located to the inner region of the ovary, and absent in granulosa cells of apoptotic follicles, and suggested a role in the recruitment of these follicles to subsequent cycles. Also, a differential expression of α-catenin and E-cadherin in some cell types was associated to molecular changes in cell–cell adhesion associated with ovulation and luteinization (Sundfeldt et al., 2000).

The expression of E- and N-cadherin has also been studied in the ovarian surface epithelium. In the mouse model, N-cadherin expression was reported (Mora et al., 2012), assigning the adhesion protein a key regulatory role on follicular structure and development (Machell and Farookhi, 2003; Zhu et al., 2004; Mora et al., 2012). Contrasting with these findings, E-cadherin expression in these cells has been found controversial; while there are reports indicating E-cadherin presence in rat, porcine and mouse ovarian surface epithelium (Ryan et al., 1996; Machell et al., 2000; Mora et al., 2012), other studies showed no E-cadherin immunoreactivity in these cells (Sundfeldt et al., 1997; Zhu et al., 2004).

Some evidence on the expression of E-cadherin had been reported in oocytes of some species, including man, rat and mouse (Campbell et al., 1995; Ohsugi et al., 1996; Rufas et al., 2000; Ziv et al., 2002, De Vries et al., 2004; Mora et al., 2012). In the murine oocyte, maternal E-cadherin and α- and β-catenin were detected at the mRNA and/or protein levels and found already assembled into a protein complex (Ohsugi et al., 1996). Regarding β-catenin, it was reported as a major tyrosine-phosphorylated protein during mouse oocyte maturation and preimplantation development (Ohsugi et al., 1999). Later studies described expression of E-cadherin at all stages of follicle development, and its co-localization with ZO-1, afadin and N-cadherin, results that led to suggest the existence of heterophilic interaction between cadherins (Mora et al., 2012).

In the follicular cells, one marked feature of cadherin expression is the absence of E-cadherin on the membrane granulosa of large preantral and antral follicles and its presence in theca cells (Machell et al., 2000). By transmission electron microscopy, junctions observed between granulosa cells and between granulosa cells and oocyte were identified as adherent junctions by expression of N-cadherin and nectin 2 and by the lack of tight junctions and desmosome-associated proteins. In a study designed to assess the relevance of cadherin-mediated intercellular interactions, it was found that whereas small follicles maintained contact between oocytes and granulosa cells under Ca^{2+}-free conditions, primary and multilayered follicles did not retain contact between granulosa cells and oocyte, indicating that nectin alone cannot maintain attachment between these two cell types, and cadherins would sustain cell–cell adhesion. In granulosa cells, N-cadherin expression has also been shown to be prominent in cells surrounding preovulatory follicles, and was found to co-localize with β-catenin (Mora et al., 2012). Homophilic N-cadherin binding has been found to promote ovarian cell (both granulose and surface epithelial cells) viability by mechanisms that involve FGFR signaling, Ca^{2+} and cAMP levels (Trolice et al., 1997; Peluso et al., 1996; Makrigiannakis et al., 1999).

E- and N-cadherin have also been related with the ovary luteinization. During this process, a decrease in N-cadherin transcript levels has been associated with increased E-cadherin expression by luteinizing granulosa cells, which would promote cell adhesion leading to the consolidation of the *corpus luteum* (Machell and Farookhi, 2003).

Sperm transport through the female tract: the sperm reservoir

Once spermatozoa are deposited in the female reproductive tract, they initiate a long journey until they reach and interact with the *cumulus*-oocyte complex vestments. Among the events that

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occur during sperm transit to the fertilization site, sperm adhesion to the oviductal epithelium appears to be essential for successful fertilization. Only thousands out of millions of spermatozoa initially deposited into the vagina arrive to the oviductal isthmus and form a functional sperm reservoir by interacting with the oviduct epithelial cells until ovulation occurs (Hunter, 2008; Suarez, 2008). Intact spermatozoa are trapped in the oviduct by the interaction between the sperm head plasma membrane at the acrosomal region and the ciliar surface of the epithelium (Hunter, 1984; Hunter et al., 1991; Lefebvre et al., 1995, Suzuki et al., 1997; Sostaric et al., 2008).

Changes associated to ovulation induce the release of oviduct epithelial cell-bound spermatozoa, allowing them to proceed with their transit to the site of fertilization in the *ampulla* (Hunter, 2008; Sostaric et al., 2008). Sperm dissociation from the epithelium of the oviduct has been related to the capacitation process (Demott and Suarez, 1992; Lefebvre and Suarez, 1996; Fazeli et al., 1999). In particular, the release has been associated to capacitation-related events well characterized in sperm cells subjected to *in vitro* incubation under defined conditions, among them changes in the sperm membrane composition and fluidity (Gadella et al., 2008) and in protein tyrosine phosphorylation (Chamberland et al., 2001) as well as the increase in intracellular Ca^{2+} levels (Handrow et al., 1989).

In vitro studies using oviduct epithelial cell monolayers have been a useful tool to determine the role of the oviductal cells in sperm selection and maintenance of the adhesion, as well as in their release (Gualtieri and Talevi 2000; Gervasi et al., 2009). These studies have demonstrated the ability of the oviduct epithelial cells to select a population of morphologically normal/non-capacitated/acrosome-intact spermatozoa (Lefebvre and Suarez, 1996; Fazeli et al., 1999; Gualtieri and Talevi, 2000). They have also shown that sperm release from the oviduct epithelial cells in culture is linked to molecular events associated with sperm capacitation (Gualtieri and Talevi, 2000; Gualtieri et al., 2005).

The expression of E-cadherin and N-cadherin was reported in the mouse and human oviductal epithelium (Tsuchiya et al., 2006; Mora et al., 2012). In the human, both molecules were found to be expressed in the endometrium, but with different profiles. E-cadherin immunoreactivity was observed throughout the menstrual cycle, primarily associated to the luminal and glandular epithelial cells (Tabibzadeh et al., 1995; van der Linden et al., 1995; Tsuchiya et al., 2006; Vazquez-Levin et al., unpublished). N-cadherin expression has been reported to vary between the proliferative and secretory phases, showing high and low expression levels, respectively (Tsuchiya et al., 2006). These molecules would be involved in tissue shedding and bleeding during menstruation (Tabibzadeh, 1996).

A recent publication from our group reported immunodetection of E-cadherin and β-catenin in bovine oviduct tissue sections and in oviduct epithelial cell monolayers and described the significant inhibitory effect of sperm incubation with an anti E-cadherin antibody upon sperm binding to oviduct epithelial cell cultures (Caballero et al., 2014). Contrasting with these observations, no expression of N-cadherin was detected the oviduct epithelium (Vazquez-Levin et al., unpublished).

There are studies suggesting the involvement of species-specific carbohydrate recognition in sperm–oviduct interaction (Lefebvre et al., 1997; Suarez et al., 1998; Suarez, 2001; Kon et al., 2009). Moreover, some proteins have been proposed to play a role in the formation of the oviductal sperm reservoir, among them other adhesion proteins (spermadhesin AQN1 in pig: Ekhlasi-Hundrieser et al., 2005). Additional evidence will be presented in the next section in support of E-cadherin participation in this event, as part of the complex array of proteins that assure sperm association to and later release from the oviductal epithelium.

E- and N-cadherin and related molecules of the adherent complex in mammalian gametes and their participation in fertilization

Presence of classical cadherins has been reported in the gametes of some mammalian species. Previous evidence has been published on immunodetection of E-, N- and P-cadherin in human, murine and rat sperm cells and oocytes (see Table 1 and references therein).

Investigations from our group thoroughly evaluated the molecular protein forms of E-cadherin, N-cadherin and related molecules in the human, bovine and murine gametes, and assessed their localization under different experimental conditions that resemble sperm transport through the female tract and interaction with the *cumulus*-oocyte complex vestments. Additionally, the involvement of both adhesion proteins in fertilization-related events has been addressed by means of well-established gamete interaction assays in the presence of E- and N-cadherin blocking antibodies.

E-cadherin was mainly localized to the flagellum and acrosomal region of non-capacitated and capacitated acrosome-intact human ejaculated spermatozoa. In human sperm cells that had undergone the acrosomal exocytosis, E-cadherin remained associated to the sperm head (Marín-Briggiler et al., 2008). In murine epididymal spermatozoa, E-cadherin was localized to the flagellum, the acrosomal and post-acrosomal regions of non-capacitated and capacitated acrosome-intact cells, and was lost in the acrosomal region of acrosome-reacted cells (Veiga et al., unpublished) (Fig. 2).

In ejaculated acrosome-intact bovine spermatozoa, E-cadherin localized to the flagellum, the apical ridge and acrosomal cap and post-acrosomal region (with differential signal intensity). Interestingly, the acrosomal signal was partially lost in sperm cells incubated under *in vitro* capacitating conditions, but remained in the apical ridge and proximal post-acrosomal region. After the occurrence of the acrosomal exocytosis, E-cadherin signal was mainly associated to the inner acrosomal membrane of bovine spermatozoa (Caballero et al., 2014) (Fig. 2).

Changes in E-cadherin localization observed in bovine spermatozoa incubated under defined conditions to promote capacitation were also found in gametes released after addition of heparin to the sperm–oviduct epithelial cell co-cultures (Caballero et al., 2014). The mechanisms that result in sperm release from the oviduct are still unknown. The shift in E-cadherin localization could be associated with heparin induced increase in intracellular Ca^{2+} ions (Medeiros and Parrish, 1996), causing E-cadherin relocalization and/or proteolysis, as reported in somatic cells (Pey et al., 1998; Ito et al., 1999). Alternatively, E-cadherin and/or β-catenin phosphorylation by kinases (i.e. Src or Casein kinases; Daugherty and Gottardi, 2007) could affect the sperm–epithelium cell adhesion stability. In this regard both protein tyrosine phosphorylation (Chamberland et al., 2001) and increase in intracellular Ca^{2+} levels (Handrow et al., 1989) have been reported during sperm capacitation. Moreover, expression of Src (Bordeleau and Leclerc, 2008) and casein kinases (Chaudhry et al., 1991a, 1991b) have been reported in bull spermatozoa. These molecular events could contribute to the sperm reservoir disassembly, allowing the male gametes to continue their journey to accomplish fertilization.

With regard to N-cadherin, preliminary studies had described detection of the transcript in ejaculated human spermatozoa (Goodwin et al., 2000), as well as the immunodetection of the adhesion protein in human and rat ejaculated spermatozoa and oocytes (Rufas et al., 2000; Ziv et al., 2002). However, its fate after sperm capacitation and acrosomal exocytosis, as well as its involvement in gamete interaction had not been characterized. Our studies thoroughly evaluated its expression, reporting the

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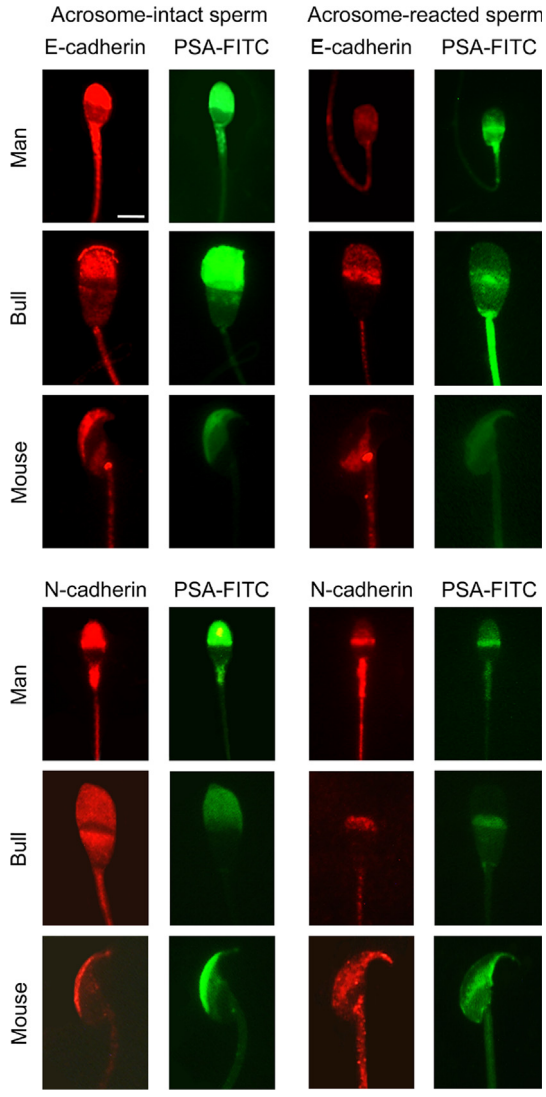

Fig. 2. Immunolocalization of E-cadherin and N-cadherin in acrosome-intact and acrosome-reacted human, bovine and murine spermatozoa. Human and bovine ejaculated spermatozoa and *cauda* epididymal spermatozoa from CF1 mice were used. Motile cells were processed for immunocytochemistry immediately or incubated under capacitating conditions and exposed to agents that induce the acrosomal exocytosis (10 μM Ca^{2+} ionophore A23187 for human and murine spermatozoa and 100 μg/ml lysophosphatidylcholine for bovine spermatozoa). Cells were stained with the anti E-cadherin (H-108, Santa Cruz Biotech., Santa Cruz, CA, USA) or anti N-cadherin (H-63, Santa Cruz Biotech.) antibody and CY3-labeled secondary antibody (Chemicon-Millipore, Billerica, MA, USA), followed by incubation with the lectin *Pisum sativum* agglutinin labeled with FITC (FITC-PSA) to assign the sperm acrosomal status. A green fluorescent signal on the acrosomal region is indicative of an intact acrosome. Bar = 2.5 μm.

transcript and protein forms in ejaculated spermatozoa. In addition, they characterized its localization in the entire flagellum and acrosomal region of testicular and ejaculated acrosome-intact human spermatozoa (Marín-Briggiler et al., 2010). In agreement with these findings, N-cadherin was immunodetected in the acrosomal and post-acrosomal regions of acrosome-intact non-capacitated and capacitated bovine spermatozoa (Vazquez-Levin et al., unpublished) and in the acrosomal region of murine sperm cells (Veiga/Marín-Briggiler et al., unpublished). In acrosome-reacted spermatozoa of the three species, N-cadherin signal was mainly associated to the equatorial segment (Fig. 2).

Since E- and N-cadherin participate in cell–cell homophilic and in some cases heterophilic interactions, their presence in the female gamete was investigated. Some evidence on the expression of E-cadherin had been reported in oocytes of some species, including man, rat and mouse, as mentioned before. Studies published from our group confirmed the expression of E-cadherin and N-cadherin in the human oocyte, and also described their presence in hamster oocytes (Marín-Briggiler et al., 2008; Marín-Briggiler et al., 2010). Our group also characterized E-cadherin and N-cadherin protein forms and their immunolocalization in the bovine *cumulus*-oocyte complexes, in both immature and mature oocytes and *cumulus* cells (Caballero et al., 2014; Vazquez-Levin et al., unpublished). Moreover, both adhesion molecules were immunodetected in murine *cumulus* cells and oocytes (Veiga et al., unpublished).

Presence of other members of the adherent complex was also investigated in both sperm and oocytes of the three species. Immunodetection (protein forms and localization in whole cells) of β-catenin and actin (total and filamentous) was also evaluated, finding that both proteins accompanied E-cadherin distribution, in favor of the presence of a functional adhesion complex on the surface of both gametes.

Localization of both E- and N-cadherin in the sperm head as well as in the oocyte surface in subcellular regions involved in gamete interaction prompted us to test their participation in adhesion events of fertilization. In the human model, the ability of sperm to fuse and penetrate an oocyte was evaluated by the heterologous sperm penetration assay (SPA) that uses human spermatozoa and *zona pellucida*-free hamster oocytes (World Health Organization, 2010). Gamete preincubation with either anti E- or anti N-cadherin specific blocking antibodies inhibited human sperm penetration of hamster oocytes in the SPA assay, suggesting that both E-cadherin and N-cadherin adhesion proteins participate in human sperm–oolemma adhesion leading to gamete plasma membrane fusion (Marín-Briggiler et al., 2008, 2010). Similar results were reported for E-cadherin by another group using a heterologous human-murine system (Purohit et al., 2004). In addition to these evaluations, sperm preincubation with anti E-cadherin but not with anti-N-cadherin specific blocking antibodies resulted in a significant impairment of sperm interaction with homologous *zona pellucida* in the human model by means of the hemizona assay (Marín-Briggiler et al., 2008); N-cadherin present in the acrosomal region may have a role in germ cell–Sertoli cell interaction and/or sperm interaction with somatic cells from the male and female reproductive tracts, but the molecular mechanisms remain to be unraveled.

To evaluate the role of cadherins in homologous gamete interaction, the bovine and murine models were used. In agreement with the studies in the human model, gamete pre-incubation with anti E-cadherin (Caballero et al., 2014; Veiga et al., unpublished) and N-cadherin (Vazquez-Levin et al., unpublished; Veiga/Marín Briggiler et al., unpublished) antibodies impaired sperm–oolemma interaction in both cases. The anti-E-cadherin blocking effect upon sperm–*zona pellucida* interaction found in the human model was also observed in the bovine (Caballero et al., unpublished) and mouse (Veiga et al., unpublished) animal models.

In conjunction, the evidence gathered suggests the expression of members of the adherent complex in the mammalian gametes and the participation of classical E- and N-cadherins in sperm interaction with the *zona pellucida* and the oolemma during fertilization-related events. Despite the fact that antibodies used by our group in protocols to block E- and N-cadherin mediated gamete (and sperm–oviduct epithelial cells) interaction have been previously reported to specifically block somatic cell–cell adhesion (and, when possible, several antibodies directed to different protein regions were used), inhibition caused by steric hindrance cannot be ruled out. Additional approaches involving specific blocking peptides as well as oocytes lacking E-, N- or both cadherins obtained from conditional knock out models will contribute to confirm some of these findings. In any case, our results are in line with recent studies

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reporting the role of the adaptor protein β-catenin in sperm–oocyte interaction, and the requirement of its degradation for gamete fusion (Takezawa et al., 2011).

Participation of E- and N-cadherin adhesion proteins in gamete interaction may imply the formation of cadherin homo (E-E, N-N-cadherin) or hetero (E-N, E-other, N-other proteins) *trans*-dimers. In support of this possibility, previous studies have shown the ability of E-cadherin to interact with N-cadherin (Volk et al., 1987) and with α2β1 (Whittard et al., 2002) and αEβ7 (Cepek et al., 1994) integrins; moreover, N-cadherin may interact with R-cadherin (Shan et al., 2000) and C-cadherin (Prakasam et al., 2006).

As mentioned for the interaction between spermatozoa and oviduct epithelial cells, gamete adhesion mediated by cadherin proteins may be regulated by protein relocalization/proteolysis caused by Ca^{2+} influx (Pey et al., 1998; Ito et al., 1999) and post-translational modifications of the adhesion complex proteins, such as Ser-Thr and Tyr phosphorylation mediated by Src and casein kinases (Matsuyoshi et al., 1992; Lickert et al., 2000; Daugherty and Gottardi, 2007; Dupre-Crochet et al., 2007). Specifically regarding Src kinases, their expression has been reported in spermatozoa of several species (human: Mitchell et al., 2008; Ruzzene et al., 1992; bovine: Bordeleau and Leclerc, 2008; mouse: Goupil et al., 2011) and very recently in oocytes (Kinsey, 2014). In addition to these mechanisms, dynamic interactions between members of the adherent complex and actin filaments appear to be crucial in remodeling and function; in this regard FXDY5/Dysadherin has been found expressed in somatic cells acting as a negative modulator of E-cadherin mediated cell adhesion by competing with the adhesion protein for the actin cytoskeleton (Nam et al., 2007). Studies performed by our group described for the first time the expression FXDY5/Dysadherin (transcript and protein) in whole testis as well as its localization in the acrosomal cap in human testis spermatids and in testicular spermatozoa. In addition, our studies showed co-localization of FXDY5/Dysadherin and E-cadherin in the head of ejaculated spermatozoa (Gabrielli et al., 2011), leading us to propose a regulatory role of sperm FXDY5/Dysadherin upon E-cadherin adhesive function following a similar mechanism described for somatic cells. Whether these mechanisms are involved in the regulation of E- or N-cadherin mediated cell membrane interactions between spermatozoa and cumulus cells and/or spermatozoa and the oocyte during fertilization-related events is currently under investigation.

Concluding remarks

Epithelial and Neural cadherin are cell–cell adhesion molecules extensively studied in numerous somatic cells and tissues in physiological and pathological conditions. This report has presented a summary of contributions from numerous researchers in the reproductive biology field demonstrating the expression of both adhesion proteins in the mammalian testis and ovary and evidence in favor of their presence in spermatozoa and cells from the *cumulus*-oocyte complex. Investigations from our group have contributed to characterize their localization in both gametes and to support their involvement in sperm interaction with the oviduct epithelial cells and between gametes in fertilization-related events. Future work will contribute to better understand the intracellular mechanisms triggered by both Epithelial and Neural cadherin-mediated cell–cell interaction events and their relevance in mammalian fertilization.

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