Ion/Water Channels for Embryo Implantation Barrier

Successful implantation involves three distinct processes, namely the embryo apposition, attachment, and penetration through the luminal epithelium of the endometrium to establish a vascular link to the mother. After penetration, stromal cells underlying the epithelium differentiate and surround the embryo to form the embryo implantation barrier, which blocks the passage of harmful substances to the embryo. Many ion/water channel proteins were found to be involved in the process of embryo implantation. First, ion/water channel proteins play their classical role in establishing a resting membrane potential, shaping action potentials and other electrical signals by gating the flow of ions across the cell membrane. Second, most of ion/water channel proteins are regulated by steroid hormone (estrogen or progesterone), which may have important implications to the embryo implantation. Last but not least, these proteins do not limit themselves as pure channels but also function as an initiator of a series of consequences once activated by their ligand/stimulator. Herein, we discuss these new insights in recent years about the contribution of ion/water channels to the embryo implantation barrier construction during early pregnancy.

Successful implantation of the human embryo is a critical event during human reproduction. Recently, it was proposed that defection of ENaC, a sodium channel, in the endometrial epithelium increases the chance of spontaneous miscarriage and implantation failure in IVF (105). Accordingly, it is implicated that ion/water channels, as pore-membrane proteins, may play critical roles in reproduction. Ion/water channels are key components in a wide variety of biological processes that involve rapid changes in cells, including establishing a resting membrane potential, shaping action potentials, and other electrical signals by gating the flow of ions across the cell membrane (46). Except for that, the functions of ion/water channels on reproduction, especially on embryo implantation, have been reported in various aspects, which have significant implications for reproduction medicine. Here, we do not only want to describe the relationships between ion/water channels and the embryo implantation barrier (EIB) but also give an extensive discussion on the progress of embryo implantation research in recent years.

Implantation of the Embryo

Embryo implantation is a complex process involving numerous genes, miRNAs, and proteins in the endometrium (21, 66, 70, 71, 95, 147). After mating, the fertilized one-cell embryo grows mitotically to reach the blastocyst stage as it enters the uterus. Implantation of the embryo has three stages: apposition, attachment, and penetration. During the stage of apposition, the trophoblast (Tr) becomes juxtaposed to the luminal epithelium (LE) sufficient to prevent slippage of the blastocyst from the flushing of the uterine lumen. During the attachment phase, the blastocyst attaches to the LE. Finally, the embryo penetrates the LE and basal lamina, and then sets up the connection between the embryo and the uterus through vascular system. During this period, stromal cell begins the process of differentiation into decidual cells, or decidualization, which leads to the transformation of the LE at the attached site of uterine lumen. The molecular details of each of these processes are not fully understood despite much endeavor in recent years (2, 6, 22, 29, 44, 68, 135).

Decidualization is the postovulatory process of endometrial remodeling in preparation for pregnancy, which also remains poorly understood (41). A functional consequence of decidualization is that the uterus transiently becomes receptive to embryo implantation. This interval is labeled as the “window of implantation” (WOI) (30). The WOI
starts ~6 days after ovulation and lasts for ~4 days in human (12). At the end of WOI, the endometrium becomes refractory to implantation, accompanying with the morphological transformation of endometrial stromal cells (ESC) into secretory epithelioid decidual cells (14). In this phase, epithelial glands, leukocytes, and arteries as well as stromal cells undergo significant morphological changes. Accompanying these changes, the transformed endometrial stromal cells form the EIB by surrounding the implanting blastocyst (124). They also provide nutritional support to the developing embryo before the establishment of a functional placenta (1). Successful embryo attachment and penetration into epithelium of the endometrium trigger decidualization and ensure the establishment of the EIB.

**EIB**

Luminal epithelial cells are the first contact sites of fetal cells with the maternal cells in the process of implantation, which takes place at midnight of day 4 or early morning of day 5 of pregnancy in mice and, 5–9 days after ovulation of the luteal phase of the menstrual cycle in humans (13, 28, 74, 137). Accordingly, they undergo a unique “plasma membrane transformation,” allowing the blastocyst to penetrate from their apical surface (85–87, 96). Accompanying embryo implantation, there are elaborate physiological mechanisms for defending the semi-allogenic embryo against the attacks from the maternal microenvironment (27, 60, 83). As the zona pellucida eventually disappears from the blastocyst before implantation and the uterine LE transforms at the stage of embryo attachment, the embryo becomes more and more vulnerable. The EIB refers to the zone of endometrial stromal cells surrounding the implanting embryo during the early period of embryo implantation (7, 136). In functional terms, it provides a barrier to prevent the passage of harmful substances to the embryo (136).

The formation of the decidua in the stromal bed surrounding the implanting embryo requires the orchestrated interaction of many cells, including transformed luminal epithelium and decidualizing stromal cells (32, 82). Stromal cells underlying the luminal epithelium begin to decidualize when the blastocyst starts to invade the luminal epithelial layer, and the most obvious feature during which is the stromal cell transformation. The small spindle-shaped stromal cells in the follicular phase transform into large plump decidual cells, with at least a five times increase in cell size. After the standard haematoxylin and eosin (H&E) stain, the decidual cells exhibit a characteristic open vesicular nucleus and pale staining cytoplasm (59). By transformation, the increased decidual cells surround the embryo and progressively generate a deciduum (37, 81, 120). The deciduum regulates the uterine microenvironment to enable embryonic growth and functions as a partial, protective barrier for the embryo. The deciduum has two distinct regions termed the primary decidual zone (PDZ) and the secondary decidual zone (SDZ), respectively. PDZ, transformed by endometrial stromal cells in the antimesometrial side of the uterus, is a densely packed avascular region surrounding the LE at the site of implantation. SDZ, next to PDZ, is proliferated and formed by the cells outside the PDZ (92, 103, 116, 131). The PDZ results in the relative isolation of the luminal epithelium from its blood supply and has incomplete tight junctions between cells. It is therefore an epithelioid tissue (142). The PDZ restricts the passage of immunoglobulins, cell, nutrients, and other substances from maternal circulation to the embryo between days 6 and 8 of pregnancy (92, 131).

The decidua impedes the movement of invasive trophoblast both by generating a local cytokine milieu that promotes trophoblast attachment and by forming a physical, permeable barrier to cell penetration. Both mechanisms protect the mother from the intrusion of trophoblasts into the uterine spiral arteries (62). So, a key function for decidua is to control the degree of invasion of the trophoblast into the maternal arteries. If this balance is maintained, it enables sufficient delivery of fetal nutrients in new blood vessels without penetrating too deep into the uterus to be detrimental to the mother.

The molecular mechanisms involved in triggering the onset of decidualization are poorly understood, although progesterone has a central role in the process by acting on estrogen-primed tissues (41). Endometrial stromal cells (ESC) differentiate into decidual cells, with a large and rounded appearance, that secrete a variety of phenotypic molecules including prolactin (PRL), IGF binding protein-1 (IGFBP-1) (73, 130), growth factors, cytokines (31, 106), proteinases for extracellular matrices, peptide hormones (9, 40, 125, 141, 143), and prostaglandins (PGs) (16, 54, 90). In these processes, the uterine LE is committed to transmitting deciduogenic signals to the stromal cells (36, 98, 115), and ion and ion channels are documented to contribute to these processes.

**Ion Channels in Endometrium and the EIB**

During implantation, the uterine fluid undergoes dynamic changes, with marked absorption in the pre-implantation period that causes closure of the uterine lumen (88, 112). This process enables the
embryo to be held in apposition with the uterine epithelium before the attachment and penetration phases. Human uterine fluid has a distinctive ionic composition compared with serum (17). The concentrations of both sodium ([Na⁺]) and calcium ([Ca²⁺]) in uterine fluid are low, whereas that of potassium ([K⁺]) increases sixfold compared with serum (80). Chloride concentrations are similar in uterine fluid and serum. The dramatic difference in uterine fluid may be largely due to the epithelial ion channels in the endometrium. Many ion and water channels are involved in the formation of the implantation barrier and contribute to the success of embryo implantation (FIGURE 1) (51, 54, 56, 60, 107, 138).

Calcium, Calcium Channels, Calcium Transporter and Binding Proteins

The entire uterine epithelium must undergo preparative remodeling because attachment of the blastocyst may occur at any point along the length of the uterus. Calcium-mediated processes play a major role in these events. In fact, Ca²⁺ is involved in many cellular signal transduction pathways as well as regulation of cell adhesion (15, 24, 35, 52, 133), which is necessary for the physiology process of endometrial epithelial cell transformation and stromal cell decidualization during embryo implantation.

The contribution of calcium ions to successful decidualization is confirmed in mice (25). In the lectin-concanavalin A-induced (Con-A) pseudopregnant animals, Con A binds to epithelial surface glycoproteins and induces the production of a decidual response in the uterine lumen (108, 109), which is undiscerning compared with that induced by blastocyst during implantation (107). In Con A-induced deciduogenic mice, luminal Ca²⁺ can facilitate the induction of the decidual reaction by affecting the LE metabolism.

Luminal decidual cells synthesize and release decidual prolactin (dPRL), which is closely linked to the process of decidualization. As the marker of decidualization, it is responsible for the coordination of the establishment and maintenance of pregnancy via entry into the circulation or locally (cytokines) through juxtacrine, paracrine, and autocrine modes of action (25, 50, 113, 126). So, long-term dPRL release is a key event for decidualization and implantation. Increasing external calcium ion concentrations does not induce the short-term secretion of dPRL in human decidual cells, but external calcium ions can produce their effects by promoting long-term release of dPRL (25). This modulates the size of the releasable hormone pool via effects on cell protein synthesis mechanisms in general. To summarize, Ca²⁺ leads to long-term dPRL release via acting on total protein synthesis rate but not on the specific modification of dPRL synthesis in human decidual cells (25).

The mechanisms underlying regulation of calcium levels in uterine tissue remain largely unknown. In the uterus, calcium ions enter into the cytoplasm through ion transport proteins, calcium ion exchangers, or calcium-binding proteins, and transient receptor potential. The transient receptor potential (TRP) protein belongs to the non-voltage-gated, Ca²⁺-permeable cation channel superfamily, including TRP canonical (TRPC), TRP melastatin (TRPM), TRP polycystin (TRPP), TRP vanilloid (TRPV), TRP mucolipin, and TRP ankyrin (38). Ovarian hormone (E2/P4)-induced TRPC1 expression is upregulated via p-CREB-mediated transcription in decidual transformation of cultured hESC, although FOXO1 may also contribute to this process. Moreover, the upregulated TRPC1 results in consequence enhancement of SOC-mediated Ca²⁺ influx, which is a crucial process in decidualization (56).

TRPM2 is an estrogen-responsive gene containing a perfect palindromic ERE in the 3'-UTR in human endometrial cells. TRPM2 is a nonselective, cation channel permeable to Na⁺, K⁺, and Ca²⁺ on intracellular binding of ADP-ribose (ADPR) to the TRPM2 COOH-terminal domain and is therefore activated by intracellular ADPR (78). In human endometrium, TRPM2 mRNA expression does not increase during the middle and late stages of the proliferative phase when the estrogen concentration increases. However, it does increase significantly during the late secretory phase of the menstrual cycle. It is well known that estrogen and progesterone are the key factors in regulating endometrial stromal cells decidualization, and both estrogen and progesterone treatment of endometrial stromal cells accelerate TRPM2 mRNA expression. Taken together, these findings suggest that TRPM2 may play a role in endometrial decidualization (47).

Gene expression profiling in the endometrium during natural and controlled ovarian stimulation cycles in “window of receptivity” show that many membrane proteins, including TRPC6 (transient receptor potential cation channel, subfamily V, member 6, also called TRVP6) are upregulated, suggesting its role in signal transduction during endometrium receptivity (48). In fact, TRPV6 expression in uterine is widely varied across species, which is upregulated by E2 in mice and pigs but by P4 in rats (23, 58, 67). In addition, the expression of TRPV6 and PMCA1 (plasma membrane Ca²⁺-ATPase) in the human endometrium during the menstrual cycle increased at the proliferative phase (early, mid, and late) compared with the other phases (138). PMCA1 is an ATP-dependent transporter that pumps calcium out of cytosol (114, 117, 121).
These findings suggest that TRPV6 and PMCA1 in luminal and glandular epithelial cells may be critical for cell proliferation and calcium homeostasis in human endometrium. So far, it is still difficult to confirm whether TRPV6 and PMCA1 are involved in the process of embryo implantation. Although it is reasonable to hypothesize their roles in EIB construction owing to their functions in controlling Ca\(^{2+}\) homeostasis, further evidence in the future should be displayed.

Ligand-gated ion channels, P2X, are predominately permeable to calcium ions but also admit other ions, such as K\(^+\) and Na\(^+\), thereby mediating cell depolarization. P2X channels stimulate Cl\(^-\) channel upregulation and K\(^+\) secretion as well as inhibiting Na\(^+\) absorption. P2X3, E-cadherin, and tenascin (the calcium-activated adhesion proteins) are very close in location and intensity in early pregnancy, until the time of implantation on day 6 in rat, suggesting a functional link between P2X and the process of implantation. As we know, during attachment and implantation, both the uterine epithelium and the adjacent extracellular matrix (ECM) undergo extensive remodeling. The newly expressed ECM modulates cell attachment, cell shape, spreading characteristics, and migration. In mice, E-cadherin has been detected on both the blastocyst and the uterine epithelium, which may also provide migration guidance for the implanting blastocyst, as well as protection of the “semi-allogeneic” embryo from the mother’s immunological responses during the invasion phase (119). Besides, tenasin facilitates embryo penetration by disrupting uterine epithelial cell adhesion to the underlying basal lamina at the site of implantation in mice (53, 119). Furthermore, P2X\(_3\) is expressed in an identical spatial and temporal pattern as tenascin and E-cadherin in uterine epithelium in rat during implantation, suggesting the inflow of Ca\(^{2+}\) required for protein activation may be controlled by these purinergic receptors. However, the exact functions of P2X3 on the transformation of endometrial stromal cells into the EIB during implantation are still unclear.

The effects of Ca\(^{2+}\) are mediated by a host of Ca\(^{2+}\)-binding proteins that serve as Ca\(^{2+}\) sensors. Ca\(^{2+}\)-binding proteins undergo conformational changes after binding calcium ions that allow them to interact with downstream effectors. Our previous study clearly elucidated that S100A11, one of the calcium-sensing proteins, mediates the regulation of cytosolic Ca\(^{2+}\) homeostasis by promoting uptake and release of calcium ions from Ca\(^{2+}\) stores in endometrial cells. This may be a crossroad in the EGF-induced interaction of the embryo and uterus during the process of embryo implantation (72). Besides, other calcium-related proteins, such as calbindin-D28k and calbindin-D9k, are reported to make a possible contribution to the process of embryo implantation (76, 77, 110, 118). Calbindin-D9k and calbindin-D28k have an almost identical cellular localization, except that CaBP-d28k protein is expressed only in glands proximal to the

![Schematic diagram of ion/water channels participating in embryo implantation and the formation of embryo implantation barrier](http://physiologyonline.physiology.org/)
lumen but not in basal glandular epithelium. They are present predominantly in luminal epithelial cells in early pregnancy in human, rat, and mouse (77). Further studies reveal that, in mouse, the level of calbindins is significantly lowered as pregnancy progressed, although expression of calbindins is increased in the early pregnant uterus compared with the nonpregnant uterus (77, 89, 111). Importantly, implantation is blocked when both CaBP-d9k and CaBP-d28k are absent in mice, and it is possible that a threshold of calbindin concentration must be reached in the uterine epithelium for successful implantation in mice (77). It is hypothesized for the role of endometrial calbindins in implantation that the increased calbindins increase the storage capacity for Ca²⁺ in the luminal epithelial cells without any harm (Ca²⁺ are bound to calbindins); but the subsequent specific down-regulated calbindins at implantation sites would release the bound Ca²⁺, leading to an increase in free Ca²⁺ concentration, which triggers the apoptosis in these specific epithelial cells, and in turn could destabilize the epithelial barrier at the implantation site and facilitate trophoblast invasion and implantation (77). However, these hypotheses still need to be substantiated in further study.

**Sodium, Sodium Channels, and Associated Proteins in Embryo Implantation**

As an common phenomena of many species, the luminal fluid disappears in the pre-implantation period, which enables the embryo to be held in contact with uterine epithelium before initiation of the implantation. Na⁺ conductance in the apical membrane of epithelia is essential for fluid absorption. Previous biophysical studies on endometrial epithelial cell layers have indicated an inward short-circuit current under basal (unstimulated) conditions. This current displays properties consistent with a Na⁺ absorption process at their apical surfaces (79) mediated by amiloride-sensitive mechanisms, which may partially explain the reasons for the generation and maintenance of the relatively low concentrations of sodium ions in human uterine fluid in vivo (17).

The amiloride-sensitive epithelial sodium channel (ENaC), encoded by SCNN1 genes, localize in the apical membrane of a wide variety of epithelia, including endometrial epithelium. ENaC contributes to electrolyte and water reabsorption (57). Notably, mechanical stresses and serine protease not only promote prostaglandin release but also activate ENaC (39, 55, 61, 134). It is documented that endometrial ENaC expression is upregulated during the peri-implantation period in mice. Endometrial ENaC expression pattern in mated mice differs from that of unmated ones, showing a great increase on days 3 and 4 after mating and a decline in expression afterward. In rodents, the expression of ENaC has been demonstrated to be upregulated during diestrus, even when progesterone levels are high (19). Additional reports describe that progesterone can simulate ENaC expression in mouse endometrial epithelial cells (129). The differences in the expression pattern between the mated and unmated animals suggest that the signaling substances released from embryos or the physical presence of the embryo may also play role(s) in the expression regulation of these ion channels.

The function of ENaC in endometrium is not limited to lumen fluid absorption but extends to the decidualization. Activation of ENaC in endometrial epithelial cells (EECs), which are sensitive to mechanical stimulation and proteases, may cause membrane depolarization, induce Ca²⁺ influx, and ultimately promote PGE2 release from epithelium. The level of ENaC expression in human endometrium before in vitro fertilization (IVF) treatment is markedly lower in women with implantation failure compared with those with successful pregnancy. These observations provide clear evidence for direct links between ion channels and stromal decidualization/embryo implantation, in addition to its role in achieving maximal uterine fluid absorption during the apposition phase (139).

**Potassium Ions, Potassium Channels, and Associated Proteins in Embryo Implantation**

Potassium ions have the primary function of generating membrane potentials. There are a wide variety of K⁺ ion channels that include the voltage-gated, Ca²⁺-activated, and inward rectifier channels (8, 26, 45, 132). It was confirmed two decades ago that the relatively high concentration of K⁺ in intrauterine fluid is required for the viability and fertilizing capacity of spermatozoa (17, 80, 99) and that high [K⁺] may enhance the cleavage rate of preimplantation embryos (100), which is in part determined by the activity of potassium channels. The most recent data demonstrate that mRNA expression of the two pore potassium (K₂P) channel subtypes (TWIK-1, TWIK-2, TRET-1, TRET-2, TASK-1, TASK-2, TASK-3, TRAAK, TALK-1, TALK-2) is seen throughout the proliferative and secretory phase of the menstrual cycle in human endometrium, with TRET-1 expression significantly greater in the proliferative phase (93). Gene profiling by microarray analysis has partially discovered the trends of potassium channel expression in specific phases of menstrual cycle in endometrium,
demonstrating a marked upregulation of cDNA for the sulfonylurea receptor (a key accessory protein of the ATP-sensitive K⁺ channel) during the window of implantation, and the high expression of gene KCNJ2 (encoding an inwardly rectifying K⁺ channel, Kir2.1) in proliferative phase along with KCNG1 (encoding Kv6.1) (123). Still, comparatively little is known of the contributions of potassium channels to EIB.

Based on our laboratory’s work in 2012 (146), expression level of BKCa (large-conductance calcium-activated potassium channels) at midsecretory phase in endometrium from infertile women undergoing IVF-ET correlates with pregnancy outcomes (146). Activation of BKCa channels induce membrane hyperpolarization, elevating intracellular Ca²⁺, leading to activation of intracellular Ca²⁺-dependent processes, including regulation of membrane potential, intracellular Ca²⁺ homeostasis, and activation of NF-κB in endometrial cells. More importantly, these regulations result in the synthesis and secretion of WOI factors in the endometrium, including leukemia inhibitory factor (LIF), integrin β3, DKK-1, and claudin-4, which modulate endometrial functions for the implanting embryo (65, 146).

**CFTR: Anion Channels in EIB**

Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated chloride channel and is expressed in the apical membrane of epithelial cells (75, 104). Alteration or elimination of CFTR activity implies transport defects. Mutations in the CFTR gene cause cystic fibrosis (CF), which is the most common lethal autosomal recessive disorder of Caucasians. In females with CF, thick and dense cervical mucus is observed, which constitutes a barrier for sperm penetration. On the other hand, male patients suffer from azoospermia as a result of the atrophy or obstruct of the epididymis, vas deferens, and seminal vesicles. Thus CF patients have reduced or no fertility due to the defects in their reproductive tract (18, 20, 34). Other possible causes include abnormal spermatogenesis and sperm capacitation in males and no oogenesis or ovulatory problems in females.

CFTR is normally expressed in epithelial cells, and surprisingly it is also found in the stroma of both mated and unmated uterine in mice with different time course, levels of expression, and immunoreactivity (139). This suggests its potential role in decidualization and angiogenesis during peri-implantation. The observations from rodents showed that the level of CFTR expression is related to the oestrous cycle, with higher levels in proestrus and estrus, and low levels in metestrus and diestrus (42, 101, 128). Besides, in rats, obvious evidence demonstrates that CFTR is regulated by steroid hormone in an estrogen-dependent manner in a novel uterine epithelial cell line (84, 101, 102, 128). More importantly, the human results are in accordance with the observations in rodents, suggesting the undoubted role of CFTR in female reproduction (127).

It is documented that CFTR expression is enhanced on day 3, but it is downregulated or absent during implantation. Meanwhile, CFTR in endometrial epithelium is thought to be a negative regulator of ENaC (18, 49, 140) to minimize its activity. Accordingly, the various expression of CFTR and ENaC in endometrium may be involved during uterine fluid absorption during implantation (19, 139), which may have significant implications in embryo implantation. Briefly, low CFTR expression and high ENaC expression at diestrus may lead to maximal fluid absorption to ensure the immobilization of the blastocyst. Nevertheless, the exact role of CFTR to reproduction, especially to the formation of EIB, is still unknown. So, further study is required to clearly elucidate the contribution of CFTR on the formation of EIB.

**Water Channels and EIB**

During implantation, glandular secretions and fluid shifts across endothelial and epithelial compartments help to prepare the uterus for the onset of implantation. Correspondingly, the contributions of water channels to absorption and secretion of uterine luminal fluids during implantation have been investigated (51).

Water moves across the cell not only by permeating through membrane lipid bilayer but also through water channels protein (WCPs). The WCPs family can be subdivided into three subfamilies: aquaporins (AQPs), aquaglyceroporins, and S-aquaporins. WCPs consist of six transmembrane-spanning domains and two hemichannels, each with a highly conserved NPA (asparagine, proline, alanine) motif (3–5, 10, 11, 63, 91, 97, 144, 145). As yet, the WCPs have 13 different members. AQP0, -1, -2, -4, and -5, also correspondingly named “orthodox,” “ordinary,” “conventional,” “pure,” “normal,” or “sensu strictu” aquaporins, belong to classical aquaporins and are water-selective channels permeable to water but not to small organic and inorganic molecules. AQP3, -7, -9, and -10, belonging to aquaglyceroporins, are nonselective water channels permeable to glycerol, urea, other small nonelectrolytes, and water. The remaining four members, AQP6, -8, -11, and -12, belonging to the third subfamily, are named by various authors as “S-aquaporins,” “superaquaporins,” or “unorthodox AQPs.” Nevertheless, there is
confusion regarding the present classification of WCPs. Recently, Benga proposed a revised nomenclature and classification system of water channel proteins as follows: aquaporin (AQPs) subfamily, aquaglyceroporin and glycerol facilitator (GlpFs) subfamily, and S-aquaporin (super-aquaporins or subcellular AQPs) subfamily.

Although there are distinct uterine expression patterns for AQPs during the peri-implantation period, there is little information available about water transport during implantation. AQPs 1–5 and 7–9 have been detected in uterus. Briefly, in humans, both AQP1 and -2 are expressed in endometrium. AQP1 localizes in the endothelia of capillaries and small blood vessels, whereas expression of AQP2 is menstrual cycle-dependent in the luminal and glandular epithelial cells of the endometrium, suggesting that E2 might regulate the expression of AQP2 in endometrial cells. Furthermore, our group has demonstrated that the complex of estrogen and estrogen receptor can bind to the promoter region of the AQP2 gene and promote the expression of the AQP2 gene in endometrial cells. The high expression of endometrial AQP2 at the proliferative and midsecretory phases implies that AQP2 may contribute to endometrial receptivity before embryo implantation. However, it is still unknown whether the E2-regulated AQP2 in endometrial cells contributes to embryo implantation and decidualization during early pregnancy.

Further studies report that AQP4-deficient mice are subfertile, and AQP7 is important in uterine decidualization. Also, increased expression of AQP5 occurs at the time of embryo implantation in rat uterus. Study of the ERE in the promoter region of AQP5 provides evidence for the direct regulation of AQP5 gene by estrogen, which suggests its role in estrogen-related events in the uterus. It is given as yet that AQPs may act on embryo implantation in an estrogen-dependent manner, but the exact mechanism is still unclear.

So far, it is difficult to describe the exact contributions of ion/water channels to embryo attachment and penetration, uterine luminal epithelium tranformation, and stromal cell decidualization during embryo implantation since many observations are confined to the records on expression levels of ion/water proteins in uterine. It is expected that they not only act as classical channels in establishing and shaping membrane potential, etc., but also exhibit various functions like other membrane proteins, such as triggering cellular signaling pathways via ligand-activated effects during embryo implantation. But these additional functions require further proof. Specifically, we do want to know how the ion/water channels are responsible for coordinating the process of decidualization or the linkage between the attached embryo and the mother in EIB establishment. In summary, with more understanding of the links between embryo implantation and ion/water channels, we anticipate that the unexpected functions of ion/water channels on embryo implantation and the construction of EIB will be determined in the future, which should provide new insights into the physiological basis of natural and assisted human reproduction.

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