Ion channels in the endometrium: regulation of endometrial receptivity and embryo implantation

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BACKGROUND: Although embryo implantation is a prerequisite for human reproduction, it remains a poorly understood process. The molecular mechanisms regulating endometrial receptivity and/or embryo implantation are still largely unclear.

METHODS: Pubmed and Medline literature databases were searched for articles in English published up to December 2013 with relevant keywords including ‘endometrium’, ‘Na⁺’, ‘Cl⁻’, ‘K⁺’, or ‘Ca²⁺ channels’, ‘ion channels’, ‘endometrial receptivity’, ‘blastocyst implantation’ and ‘embryo implantation’.

RESULTS: At the time of writing, more than 14 types of ion channels, including the cystic fibrosis transmembrane conductance regulator, epithelial sodium channel and various Ca²⁺ and K⁺ channels, had been reported to be expressed in the endometrium or cells of endometrial origin. In vitro and/or in vivo studies conducted on different species, including rodents, pigs and humans, demonstrated the involvement of various ion channels in the process of embryo implantation by regulating: (i) uterine luminal fluid volume; (ii) decidualization; and (iii) the expression of the genes associated with implantation. Importantly, abnormal ion channel expression was found to be associated with implantation failure in IVF patients.

CONCLUSIONS: Ion channels in the endometrium are emerging as important players in regulating endometrial receptivity and embryo implantation. Abnormal expression or function of ion channels in the endometrium may lead to impaired endometrial receptivity and/or implantation failure. Further investigation into the roles of endometrial ion channels may provide a better understanding of the complex process of embryo implantation and thus reveal novel targets for diagnosis and treatment of implantation failure.

Key words: ion channel / endometrium / endometrial receptivity / embryo implantation / implantation failure
Introduction

The implantation of the blastocyst into the uterus is a prerequisite for human reproduction. Nearly 75% of pregnancy losses in humans are believed to be due to implantation failure (Wilcox et al., 1988; Norwitz et al., 2001). Despite the advances in IVF and embryo transfer for assisted reproduction technology (ART), the pregnancy rate of ART remains low, which is largely due to implantation failure (Toth et al., 2011). In addition, defective implantation can affect the later course of pregnancy resulting in poor outcomes or disease (Cha et al., 2012). Ethical concerns and technical issues limit the analysis of human reproduction, therefore most of the studies exploring implantation mechanisms are carried out on animal models, and have revealed a large number of genes, signaling pathways and cellular interplays critically involved in embryo implantation (see reviews (Wang and Dey, 2006; Singh et al., 2011; Cha et al., 2012; Koot et al., 2012)). However, complicated as it is, embryo implantation remains a poorly understood process.

Successful implantation requires the coordination of embryo development into an activated blastocyst and uterine transition into the receptive state. As the maternal component, the uterus comprises mainly epithelial and stromal tissues that constitute the endometrium, and the outlying muscles known as the myometrium. The endometrium plays essential roles in embryo implantation and is only receptive for the blastocyst to implant for a limited period of time, the so-called ‘receptive window’ (Bazer et al., 2011; Singh et al., 2011; Revel, 2012). Although endometrial receptivity is not yet fully understood, it is well documented that a series of physiological events along with morphological changes and an altered gene expression profile in the endometrium occur during the peri-implantation period, which are believed to ensure the success of embryo implantation (Bazer et al., 2011; Singh et al., 2011; Revel, 2012).

Ion channels are a group of trans-membrane proteins allowing ions to flow across cell or organelle membranes (Hille, 1986). Ions flow through ion channels can result in cellular or organelle changes in membrane potential, ion gradients, pH and second-messenger signaling. These features make ion channels essential for many physiological processes such as neuronal signal transmission (Armstrong and Hille, 1998), muscle contraction (Balse et al., 2012), cell volume regulation (Lambert et al., 2008), acid-base homeostasis (Fischer and Widdicombe, 2006) and epithelial secretion/absorption (Chambers et al., 2007; Schild, 2010) as well as cell proliferation, apoptosis and migration, particularly related to cancer development (Pardo, 2004; Wulff et al., 2009; Cuddapah and Sontheimer, 2011; Lehen’kyi and Prevarskaya, 2011). The involvement of ion channels in many physiological processes is reflected by their tight regulation by a wide spectrum of hormones, including ovarian hormones, progesterone and estradiol (E2), and growth factors (Lang et al., 2003; Pao, 2012). In addition, the gating of ion channels is dynamic and subject to a variety of stimuli, including membrane charges (Armstrong and Hille, 1998), mechanical forces (Arnadottir and Chalfie, 2010; Zhang et al., 2010), temperature (Ferrер-Montiel et al., 2012) and chemical substances (Gadsby and Naim, 1999; Gever et al., 2006; Kleyman et al., 2009), which allows ion channels to detect a variety of extracellular or intracellular signals and transduce them into various cellular responses. Given their versatile roles and membrane localization, ion channels are often considered ideal pharmaceutical targets (Camerino et al., 2007; Verkman and Galletta, 2009).

A number of ion channels have been discovered in the endometrium both in the epithelium and stroma. While studies on endometrial ion channels are still limited, the evidence collected to date has revealed important roles for ion channels, especially the ones in the endometrial epithelium, in regulating endometrial receptivity and embryo implantation (Ruan et al., 2012; Zhang et al., 2012). In this review, we summarize these findings and make the first attempt to discuss the involvement of ion channels in the regulation of endometrial receptivity and embryo implantation. While other ion transporters, such as the anion transporter/exchanger family members, SLC4 and SLC26, have been implicated in the implantation process (Suzuki et al., 2002; He et al., 2010a; Liu et al., 2012b; Chan and Sun, 2013), they are not considered typical ion channels and are therefore excluded in the current review.

Methods

PubMed and Medline literature databases were searched for papers and books in English published up to December 2013. Keywords including endometrium, Na+, Cl−, K+, Ca2+ channels, ion channels, endometrial receptivity, blastocyst implantation and embryo implantation were used for the search. Data obtained from humans or other species, by all experimental techniques, are included in this review.

Critical events and factors influencing endometrial receptivity and embryo implantation

Implantation is a process establishing an intimate connection between the blastocyst and the endometrium, which is initiated by the blastocyst apposing closely to the luminal epithelium and followed by its attachment or adhesion to the epithelium. In species such as humans and rodents, the blastocyst is invasive enough to penetrate the epithelium after the initial attachment. A variety of factors, such as steroid hormones, morphogens, cytokines, adhesion molecules, growth factors and transcription factors, have been shown to play a role in the course of implantation and are reviewed elsewhere (Wang and Dey, 2006; Singh et al., 2011; Cha et al., 2012; Koot et al., 2012). We discuss here the major events and factors during the peri-implantation period that have been demonstrated to be, or may possibly be, associated with ion channels.

Uterine luminal fluid reduction

A pre-implantation blastocyst is tiny (<0.2 mm in diameter in humans) compared with the uterine cavity (Richter et al., 2001). Stabilizing the blastocyst in the uterine lumen to establish a close contact with the endometrium is believed to be a prerequisite for implantation. The uterine lumen contains electrolytes/water-based fluid, the volume of which is known to fluctuate throughout the menstrual cycle under the influence of ovarian hormones (Casslen, 1986; Naftalin et al., 2002; Chan et al., 2012). In humans, a significant reduction in uterine luminal fluid has been observed in the mid-secretory phase of the cycle when the endometrium is receptive for implantation (Casslen, 1986; Maier and Koslus, 1988). Similarly in rodents, the entire uterine lumen disappears at the onset of implantation resulting in complete apposition of opposing epithelial linings, the so-called ‘lumen closure’ (Martin et al., 1970; Thorpe et al., 1974; Wang and Dey, 2006). The reduction in luminal fluid is believed to minimize movement of the blastocyst and is, therefore, a key event for implantation (Salleh et al., 2005; Chan et al., 2012).
importance of uterine luminal fluid control for implantation in humans is evident by the fact that in ART clinics, the fluid volume transferred along with the embryo into the uterus is limited to 20–60 μl to ensure a successful implantation (Schoolcraft et al., 2001; Magli et al., 2008). Moreover, it has been reported that uterine fluid accumulation without ultrasound-visible hydrosalpinges during ART cycles in humans can result in a significantly lower pregnancy rate (Levi et al., 2001), suggesting that clearance of excessive uterine luminal fluid is essential for implantation in humans.

Uterine luminal fluid could originate from the influxed peritoneal fluid via Fallopian tubes since Fallopian tube ligation was reported to cause a reduction in uterine luminal fluid from 113–180 to 83–127 μl at mid-cycle in humans (Casslen, 1986). However, this amount (83–127 μl) of fluid found at mid-cycle (time of ovulation) after tubal ligation was still considerably higher than the average levels (5–35 μl) normally found at mid-secretory phase (receptive for implantation) (Casslen, 1986), suggesting that the uterus itself may play an active role in the dynamic change of uterine luminal fluid volume during the cycle. Indeed, in rodents, under hormonal influence, a predominance of fluid absorption over secretion by the endometrial epithelium results in a remarkable reduction in luminal fluid in the peri-implantation period (Naftalin et al., 2002; Salleh et al., 2005; Chan et al., 2012). Of note, the ‘secretory-phase’ in the menstrual cycle in humans is the receptive phase for implantation, which refers to the phase when endometrial glands secrete glycoconjugates, glycoproteins or lipids (Verma, 1983); however, the uterine luminal fluid is actually reduced (Casslen, 1986; Maier and Kulis, 1988), from 113–180 μl to 5–35 μl during this phase, suggesting a net fluid absorption across the endometrium during this period in humans.

A number of ion channels have been identified in endometrial epithelium (Chan et al., 2012). They transport ions, thereby building up electrolyte gradients to drive water movement, either secretion or absorption, across the epithelium (Salleh et al., 2005; Chan et al., 2009). Dysfunction of ion channels or mis-regulation of their expression has been shown to result in disorders of fluid transport and implantation failure (He et al., 2010b) (see sections below).

Decidualization and the role of the blastocyst

The process of proliferation and differentiation of endometrial stromal cells (ESCs) into large, round and growth factor-secreting decidual cells, the so-called decidualization, is essential to successful implantation and pregnancy (Ansell et al., 1974; Arias-Stella, 2002). Aberrant decidualization can result in adverse consequences such as preterm birth and fetal death (Lam et al., 2005; Dokras et al., 2006; Hirota et al., 2010; Sun et al., 2010; Cui et al., 2012). Decidualization is hormonally regulated, since in humans decidualization is present at the luteal phase during regular menstruation cycle (Gellersen et al., 2007). In addition, decidualization is influenced by the blastocyst. In humans, decidualization is significantly enhanced at blastocyst-implantation cycles compared with that during nonconception cycles (Gellersen et al., 2007; Cha et al., 2012). In rodents, the presence of a blastocyst initiates decidualization (Lee et al., 2007a). Interestingly, in pseudo-pregnant rodents decidualization is triggered by artificial stimuli such as scratching on the endometrium (Finn, 1966; Lejeune et al., 1982). Similarly in humans, implantation is prompted by intrauterine mechanical stimulation such as endometrial biopsies (Almog et al., 2010). Of note, the artificially-stimulated decidualization is precluded when the endometrial epithelium is destroyed (Lejeune et al., 1981). These observations suggest that at the onset of normal implantation, the attachment of the blastocyst to the endometrial epithelium may be a physical signal promoting decidualization. Furthermore, the invasive implanting blastocyst releases different types of proteases which are required for successful implantation and decidualization (Sawada et al., 1990; Salmansen and Nie, 2002). Interestingly, certain epithelial ion channels are sensitive to mechanical forces (Fronius and Clauss, 2008; Zhang et al., 2010) or activated by protease-mediated cleavage (Kleyman et al., 2009), which indicates the potential of ion channels in endometrial epithelium to respond to blastocyst-derived factors, and thus a possible role in regulating decidualization.

Implantation-associated gene expression

Embryo implantation is a complex process involving changes in expression of an array of genes including leukemia inhibitory factor (LIF) (Stewart et al., 1992), Indian hedgehog (Ihh) (Matsumoto et al., 2002), bone morphogenetic protein 2 (Bmp2) (Lee et al., 2007b) and homeobox proteins Hoxa10 (Lim et al., 1999) and Hoxa11 (Gendron et al., 1997). Prostaglandins (PGs), synthesized by cyclooxygenases (COXs) from phospholipase A (PLA)-derived arachidonic acids (Ruan et al., 2011), are considered to be the most important molecules for decidualization and implantation. Deletion of Plo-2 and Cox-2 genes results in infertility in mice (Lim et al., 1997; Miller and Sassoon, 1998; Li et al., 2007; Daikoku et al., 2011). However, it remains largely unclear how such a complex array of genes involved in implantation are regulated. A growing body of evidence has indicated the capacity of ion channels to regulate gene expression indirectly, through their ability to activate signaling pathways leading to the activation/inactivation of transcription factors (Mellstrom et al., 2008; Xu et al., 2011; Jiang et al., 2012; Lu et al., 2012).

Ion channels in the endometrium

Over the last two decades, a number of ion channels have been found to be expressed in the endometrium of different species, including the mouse, rat, porcine and human (Chan et al., 2012). Efforts have been made to investigate the involvement of various endometrial ion channels in different reproductive events, including embryo implantation. Although the exact roles of ion channels are far from understood, pioneering works have revealed the involvement of some ion channels in regulating endometrial receptivity and embryo implantation, abnormalities of which have been shown to result in implantation failure.

Cystic fibrosis transmembrane conductance regulator (CFTR)

CFTR in the endometrium

CFTR is a cAMP-activated anion channel belonging to the ATP-binding cassette family (Hwang and Kirk, 2013). CFTR mediates Cl⁻ efflux, driving water movement into the lumen, which is essential for epithelial fluid secretion. In humans, mutations of the CFTR gene cause cystic fibrosis (CF), a common regressive disease in Caucasians, which is characterized by defective electrolyte and fluid transport in a wide variety of epithelia and has long been noted with fertility problems in both males
and females (Jarzabek et al., 2004; Chan et al., 2009; Chen et al., 2012; Ahmad et al., 2013). Although CFTR was long observed in the endometri- nal epithelium of different species (Trezise and Buchwald, 1991; Tizzano et al., 1994; Mularoni et al., 1996; Deachapunya and O’Grady, 1998; Chan et al., 2002; Zheng et al., 2004) and demonstrated to play an active role in endometrial Cl− and fluid secretion (Gray et al., 1993; Chan et al., 1997a, 1999; Deachapunya and O’Grady, 1998; Fong and Chan, 1998; Fong et al., 1998), it is thought to play limited role in implantation since many women with CF can achieve a natural conception (Thorpe-Beeston et al., 2013). However, accumulating evidence indicates that CFTR plays a role in regulating apoptotic activity of endometri- nal epithelial cells (EECs) (Yang et al., 2011b) and that the interplay between CFTR and the epithelial sodium channel (ENaC) dynamically regulates the uterine luminal fluid volume (Chan et al., 2001), which has important bearings on embryo implantation (see sections below).

Hormone-down-regulated CFTR at implantation
The expression of CFTR in the uterus changes with the dynamics of ovarian hormones during the cycle. In mice, the maximal level of uterine CFTR was observed at estrus when E2 is predominant. At diestrus when progesterone overcomes E2, CFTR was undetectable in mouse uteri (Chan et al., 2002). Consistently, in rodent EEC cultures, E2 promoted, while progesterone attenuated CFTR expression or activity (Mahfoudi et al., 1994; Rochwerger et al., 1994; Chan et al., 2002). In humans, CFTR in the endometrium was reported to be significantly higher in the late proliferative phase compared with other phases (Zheng et al., 2004). E2-induced up-regulation of CFTR might be responsible for the increased volume of uterine fluid secretion into the lumen, as found in mice at estrus phase (He et al., 2010a) and in humans at proli- feration phase implantation (Casslen, 1986; Maier and Kuslis, 1988). This notion is further confirmed by another study showing that in ovariec- tomized mice treatment with E2 enhanced CFTR mRNA level in the uterus and uterine fluid accumulation (Nobuzane et al., 2008). Furthermore, in the peri-implantation period in mice, when progesterone is predominant over E2, CFTR was not detected in the epithelium by immunohistochemi- stry, and CFTR mRNA levels were low (Yang et al., 2004). In the receptive phase in humans, the level of uterine CFTR was also decreased (Zheng et al., 2004). The down-regulation of CFTR may contribute to the reduction in uterine fluid volume at implantation by reducing fluid secre- tion. CFTR is also known to interact with another ion channel, ENaC (Schreiber et al., 1999) and normally inhibits ENaC-mediated endometri- nal absorption (Chan et al., 2001). The down-regulation of CFTR during implantation would remove its inhibitory effect on ENaC thereby enhan- cing absorptive activity of the endometrial epithelium. Together, reduced fluid secretion and maximized absorption as a result of CFTR down-regulation in the peri-implantation period appear to contribute to the reduction in uterine fluid volume required for implantation.

Abnormal up-regulation of CFTR and implantation failure
The significance of the down-regulation of CFTR at peri-implantation is highlighted by the observed implantation failure caused by abnormal up-regulation of CFTR. Chlamydia trachomatis infection, the most common cause of pelvic inflammation, is well known for its association with tubal damage that is thought to be the major cause of female infer- tility in humans (El Hakim et al., 2010). Our recent studies have also revealed another possible reason for implantation failure in a chlamydial infection mouse model (He et al., 2010b). Intrauterine injection of C. trachomatis lipopolysaccharide in mice caused a significant decrease in implantation rate accompanied by increases in uterine CFTR, CFTR-mediated anion secretion and uterine wet weight (He et al., 2010b). These results suggest that abnormally up-regulated CFTR ex- pression may cause luminal fluid accumulation and thus implantation failure. However, this possibility remains to be confirmed in humans. A recent study has reported a decrease, rather than an increase, in endo- metrial CFTR expression in infertile patients with hydrosalpinx (Song et al., 2012). The discrepancy in findings between the chlamydial infection mouse model and infertile patients was explained, apart from the obvious species difference, by the difference in inflammatory state, being in the acute phase in the former and the chronic phase in the latter. Further investigation is required to clarify this.

Interestingly, in ART, the implantation rate is considerably reduced with controlled ovarian hyperstimulation (COH) (Simon et al., 1995; Joo et al., 2010). Yang et al. reported that the endometrial cells from preg- nant mice after COH showed higher apoptotic activity and increased CFTR expression when compared with pregnant mice that conceived without COH (Yang et al., 2011b). In the same study, treating mouse endometrial cells with high levels of E2 in vitro increased both the expres- sion of CFTR and apoptosis in these cells, which could be prevented by co-treatment with a selective CFTR inhibitor, suggesting the involvement of CFTR in regulating apoptotic activity of endometrial cells. Of note, CFTR has also been found to play a role in cell apoptosis in other tissue types (Rottner et al., 2009; Vandivier et al., 2009). Although apop- tosis of EECs is known to occur prior to decidualization/implantation (Galan et al., 2000; Tassell et al., 2000; Correia-da-Silva et al., 2004; Zhang and Paria, 2006), excessive apoptosis could be detrimental and associated with failing pregnancies in humans (Kokawa et al., 1998). Given that COH is an essential procedure to maximize ovulation for IVF, the COH-induced abnormally high expression of CFTR and thus ex- cessive apoptosis might be one of the factors contributing to the low implantation rate observed in ART clinics.

Taken together, the evidence suggests that down-regulation of CFTR at the time of implantation is necessary for ensuring success. Abnormally up-regulated CFTR during the peri-implantation period, either by bacterial infection or hormone disturbance, can result in abnormal uterine fluid accumulation or increased apoptosis of EECs, leading to implantation failure in mice. However, whether this is the case in humans requires further investigation. It should be noted that while most women with CF seem to be able to conceive, they tend to have an increased rate of preterm labor (Thorpe-Beeston et al., 2013), a condition that may result from defective implantation (Hirota et al., 2010). Detailed studies of the role of CFTR in the full spectrum of the implantation process may provide the answer.

Epithelial sodium channel (ENaC)
ENaC and uterine fluid absorption
The ENaC, also known as amiloride-sensitive sodium channel (ASSC) or sodium channel non-neuronal 1 (SCNN1), is composed of α, β and γ subunits (α-ENaC, β-ENaC and γ-ENaC) (Kellenberger and Schild, 2002). ENaC is well known for its key role in epithelial fluid absorption especially in the lung and kidney (Hummeler et al., 1996; Salker, 2010). In the endometrium, amiloride-sensitive sodium absorptive function has been observed in humans, mice and pigs (Matthews et al., 1993, 1998; Chan et al., 1997a; Vetter et al., 1997). Most recently, an
immunohistology study has shown α-ENaC expression in the apical membrane of human endometrial epithelium (Enuka et al., 2012). Together with the identification of Na+/K+-ATPase activity in the endometrium (Chan et al., 1997a; Deachapunya et al., 1999), it is generally accepted that, as in the lung and kidney, uterine luminal Na+ is absorbed by EECs via apically located ENaC and then transported into the blood via basolateral Na+/K+-ATPase. This results in a Na+ gradient across the endometrial epithelium, which provides the driving force for water absorption from the lumen into the blood.

In situ hybridization results show α-ENaC, β-ENaC and γ-ENaC gene expression in mouse uterus, which exhibits cyclic changes during the estrous cycle (Chan et al., 2002). However, in contrast to CFTR, all three ENaC subunits showed a maximal level at diestrus but lower at estrus (Chan et al., 2002), indicating that ENaC is up-regulated by progesterone while down-regulated by E2. Consistently, down-regulation of uterine α-ENaC mRNA expression was also observed with concurrent up-regulation of CFTR and uterine fluid accumulation after treating ovariectomized mice with E2 (Nobuzane et al., 2008). Also, in ovariectomized rats, treatment with E2 caused fluid secretion across the endometrium, while progesterone resulted in amiloride-sensitive fluid absorption (Salleh et al., 2005). At implantation, when progesterone is predominant, increased uterine ENaC and decreased CFTR expression was found in mice (Yang et al., 2004). We thus propose that the uterine fluid reduction necessary for embryo implantation is a result of maximal fluid absorption and minimal fluid secretion (Figs 1 and 2).

**ENaC and decidualization**

As discussed earlier, decidualization can be promoted by mechanical stimuli. Serine proteases are abundantly expressed at the blastocyst-endometrium interface. Both mechanical force and serine protease can activate ENaC (Fronius and Clauss, 2008; Kleyman et al., 2009) (Fig. 2). These gating properties of ENaC, together with its apical localization to the luminal epithelium (Enuka et al., 2012) and maximal expression at implantation (Yang et al., 2004), make ENaC a strong candidate for responding to the decidualizing signals from the implanting embryo and eliciting downstream events leading to decidualization. We conducted a series of experiments to test this possibility (Ruan et al., 2012) and showed that the highest level of cleaved α-ENaC, the documented signature of protease-induced ENaC activation (Kleyman et al., 2009), was observed in the uterus at implantation in mice. In isolated mouse EECs, trypsin, a protease that has been reported to be released by the embryo (Sawada et al., 1990), induced amiloride-sensitive whole-cell currents and membrane potential depolarization, which was abrogated by amiloride or aprotinin, a protease inhibitor, suggesting ENaC activation leading to Na+ influx upon protease stimulation. Trypsin also induced Ca2+ influx in the EECs, which was abolished by amiloride or nifedipine, an inhibitor of the voltage-activated Ca2+ channel, suggesting that ENaC-mediated membrane depolarization can lead to activation of the Ca2+ channel. The ENaC activation-induced Ca2+ increase was found to trigger the release of PGE2, the well-known decidualizing molecule (Lim et al., 1997), from the EECs. Interestingly, treatment of EECs with trypsin also caused increases in COX-2 mRNA level and phosphorylation of cAMP/Ca2+ response element-binding protein (CREB), the transcription factor known to regulate COX-2 (Tsatsanis et al., 2006). Both effects could be blocked by amiloride or nifedipine, suggesting that the activation of ENaC and the following Ca2+ increase may activate CREB-driven transcription of COX-2 and thus result in a more sustained production of PGs in EECs for decidualization. In a co-culture of mouse EECs and ESCs, trypsin-induced

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**Figure 1** Involvement of the cystic fibrosis transmembrane conductance regulator (CFTR) and epithelial sodium channel (ENaC) in regulating uterine fluid volume for implantation. Up-regulation of ENaC and down-regulation of CFTR result in maximal fluid absorption and minimal fluid secretion leading to uterine fluid reduction during implantation (left). Abnormal up-regulation of CFTR causes abnormal increase in fluid secretion and luminal accumulation resulting in implantation failure (right).
ENaC activation was shown to induce morphological change of ESCs into polygonal and multi-nuclei cells, the well-documented decidual cell morphology in vitro (Fouladi Nashta et al., 2004). These data suggest that activation of ENaC and the resulting PGE2 release from EECs led to decidualization of the co-cultured ESCs. Consistently, intrauterine injection of amiloride inhibited the oil-induced decidualization in pseudo-pregnant mice, confirming an essential role of ENaC in decidualization (Ruan et al., 2012). These results suggest that ENaC plays a key role in converting the signal from the implanting embryo, serine protease in this case, into downstream cellular responses leading to decidualization in the stroma, as depicted in Fig. 2.

The high expression level of ENaC observed during the peri-implantation period (Yang et al., 2004), together with its demonstrated roles in uterine fluid absorption (Chan et al., 1997a; Salleh et al., 2005) and signaling transduction for decidualization (Ruan et al., 2012), suggests that ENaC is required for implantation. Indeed, intrauterine injection of amiloride or α-ENaC-targeting small interfering RNA (siRNA) also caused a significant reduction in implantation rate in mice with altered expression of implantation-associated genes (Fig. 2) (Ruan et al., 2012). In humans, endometrial samples collected prior to embryo transfer during the IVF procedure showed significantly lower levels of α-ENaC and γ-ENaC protein in women with a failed pregnancy after IVF, compared with those with a successful pregnancy (Ruan et al., 2012), suggesting the crucial involvement of ENaC in implantation. Recently, it has also been reported that the key regulator of ENaC, the serum- and glucocorticoid-inducible kinase 1 (SGK1) (Fig. 2), is required for embryo implantation (Salker et al., 2012), supporting a key role of ENaC in the process. Since ENaC is also known to be activated by mechanical forces, the demonstrated role of ENaC in decidualization may also provide an explanation for the mechanically stimulated decidualization in rodents and the increased pregnancy rate in IVF patients after endometrial scratch (Almog et al., 2010).

Taken together, ENaC has been shown to be involved in (i) uterine fluid absorption and (ii) promoting decidualization, which are essential for implantation. Given its ability to activate CREB and COX-2, which regulate a number of downstream genes associated with implantation, such as peroxisome proliferator-activated receptor (PPAR)-δ, retinoid X receptor (RXR), leukemia inhibitory factor (LIF), Dickkopf-related protein 1 (DKK-1), forkhead box protein O1 (FOXO-1), insulin-like growth factor binding protein 1 (IGFBP-1), and GABA receptor, it plays a versatile role in implantation, well beyond fluid absorption and decidualization. Further investigation into its role in different processes of implantation may reveal novel causes underlying implantation/pregnancy-related disorders or diseases.

Ca²⁺ channels
It has long been acknowledged that intracellular Ca²⁺ is essential for implantation. Increases in intracellular Ca²⁺, or Ca²⁺ mobilizations, have
been implicated in a variety of processes pertinent to implantation, including the regulation of blastocyst-endometrium adhesion (Thie and Denker, 2002), heparin-binding EGF-like growth factor (HB-EGF) signaling (Wang et al., 2000) (Fig. 2), epithelial tight junctions (Denker and Nigam, 1998), protease activity (Tabibzadeh, 1996), epithelial transport (Chan et al., 1997b, 2000; Wang and Chan, 2000) and endometrial PGs production (Burns et al., 1998; Ruan et al., 2008, 2011). In addition, the Ca\(^{2+}\)-binding proteins CaBP-9k, -28k and S100P have been reported to regulate endometrial receptivity (Luu et al., 2004a, b; Liu et al., 2012a). It is also well established that intracellular Ca\(^{2+}\) that endometrial epithelium might have an active Ca\(^{2+}\) influx activity to CREB phosphorylation, COX-2 expression up-regulation and PGE2 release, which perhaps explains the differential expression of Ca\(^{2+}\) entry'. The presence of TRP channels in the endometrium was suggested by the observed sensitivity of the Ca\(^{2+}\) influx activity to SKF-96365, a reported inhibitor of TRP channels in RL95-2 cells (Thie and Denker, 2002). In addition, the E\(_2\) elicited transient Ca\(^{2+}\) influx in RL95-2 cells was significantly increased by the depletion of intracellular Ca\(^{2+}\) stores (Perret et al., 2001), consistent with the involvement of TRP channels in mediating SOC in the endometrium.

TRPC1, a member of the TRP family, has been reported to be expressed in ESCs. Kawarabayashi et al. demonstrated that the E\(_2\) or progesterone-induced decidualization in vitro was accompanied by up-regulation of TRPC1 and increases in TRPC1-mediated SOC activity in human ESCs (Kawarabayashi et al., 2012). The induced decidualizing responses in hESCs, including an increase in the size of hESCs and up-regulation of IGF-binding protein 1 and prolactin, were inhibited by siRNA targeting TRPC1, TRP channel inhibitors or an antibody against TRPC1 (Kawarabayashi et al., 2012). Translocation of phosphorylated CREB and enhanced expression of forskhad box protein I were also found in parallel with decidualization in hESCs, which could be counteracted by the siRNA targeting TRPC1 (Kawarabayashi et al., 2012). TRPC1-deficient mice are fertile (Dietrich et al., 2007), which would suggest a dispensable role for TRPC1 in implantation. Thus, the exact role of TRPC1 in the endometrium and its possible involvement in implantation remains to be elucidated.

Another TRP member, TRPV6, is expressed in the luminal and glandular epithelia in human, mouse, rat and pig endometrium. TRPV6 undergoes cyclic changes during the ovarian cycle and appears to be subject to E\(_2\) or progesterone regulation. In humans and mice, the maximal mRNA level of TRPV6 was detected in the proliferative phase or estrus, when E\(_2\) is predominant (Lee and Jeung, 2007; Yang et al., 2011a). Consistently, injection of E\(_2\) to immature mice enhanced the expression of TRPV6 in the endometrium, which could be abolished by an E\(_2\) receptor \(\beta\) antagonist (Lee and Jeung, 2007). The E\(_2\)-induced TRPV6 expression was also observed in a human endometrial cell line, Ishikawa (ISK) (Yang et al., 2011a). However, in contrast to human and mouse, rat endometrium showed highest TRPV6 mRNA expression at diestrus (Kim et al., 2006). TRPV6 is also highly expressed in the endometrium during pregnancy. In mice, TRPV6 mRNA was detected starting from Day 7 of pregnancy and peaked at mid-gestation (Lee and Jeung, 2007). In rats and pigs, the maximal mRNA level of TRPV6 was detected at implantation (Choi et al., 2009). Although no direct evidence for the involvement of TRPV6 in implantation has been reported, female TRPV6 knockout mice are subfertile, take longer to become pregnant and have a smaller litter size than wild-type mice (Bianco et al., 2007). It seems plausible that compromised implantation might be one of the causes for the reduced fertility in TRPV6 knockout mice, details of which await further investigation.

Taken together, although the importance of intracellular Ca\(^{2+}\) in implantation has been well recognized, the exact role of many endometrial Ca\(^{2+}\) channels in the process of implantation remains largely unclear except for L-type VDCC, which has been demonstrated to be the key to the ENaC-involved signaling pathway leading to stromal decidualization (Ruan et al., 2012). How all these different types of Ca\(^{2+}\) channel are orchestrated during implantation remains an open question.

**Ion channels in embryo implantation**

VDCCs

VDCCs are a group of Ca\(^{2+}\) channels activated by a depolarizing membrane voltage and are categorized into different types based on their activation and inactivation kinetics. For example, L-type VDCCs mediate relatively long-lasting Ca\(^{2+}\) influx (Lipscombe et al., 2004). Intracellularly applied blockers of VDCCs (nifedipine, verapamil, nicidepine and diltiazem) reduced the decidual response in pseudo-pregnant mice (Sakoff and Murdoch, 1994), suggesting possible involvement of Ca\(^{2+}\) channels in decidualization. To date, two categories of Ca\(^{2+}\) channels have been found in the endometrium, including voltage-dependent Ca\(^{2+}\) channels (VDCCs) and transient receptor potential (TRP) channels.

TRPC1

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K⁺ channels and uterine electrolyte and fluid transport

K⁺ channels have been found in endometrial epithelium and demonstrated to be involved in uterine electrolyte transport. Vetter et al. found a cAMP-activated, barium-sensitive and inward rectifying K⁺ channel in the basolateral membrane of porcine endometrial epithelium, activation of which stimulated Na⁺ absorption across the epithelium (Vetter et al., 1997). Fong et al. (1998) found that adrenalin-activated Cl⁻ secretion across the endometrium also depends on the basolateral K⁺ channels. These studies suggest that both Na⁺ absorption and Cl⁻ secretion depend on the activity of basolateral K⁺ channels, since the K⁺ efflux through K⁺ channels provides a recycle pathway for the K⁺ pumped into the cells by Na⁺-K⁺-ATPase (Fig. 2). K⁺ channels have also been found in the apical membrane of endometrial epithelium. Palmer et al. showed that in apical membrane of porcine endometrial gland epithelial cultures, UTP elicited a transepithelial current, which was K⁺ dependent and sensitive to an inhibitor of the small-conductance calcium-activated K⁺ channel (SK) (Palmer et al., 2008). Gene expression of two SK subtypes, SK1 and SK3, was detected by RT–PCR and found to be up-regulated by E2 in porcine endometrial cultures (Palmer et al., 2008). An early study on human endometrial epithelial monolayer cultures showed that K⁺ is passively secreted into the apical compartment (Matthews et al., 1993). This is consistent with the finding that the uterine luminal K⁺ level is higher than that in serum (Casslen and Nilsson, 1984), and the apical K⁺ channels may be responsible for the K⁺ secretion.

Implications of K⁺ channels in decidualization and implantation

The endometrium undergoes a series of programmed morphological changes or remodeling in order to accommodate the implanting embryo. Decidualization starts with proliferation in ESCs (Huet-Hudson et al., 1989; Correia-da-Silva et al., 2004). Simultaneously, luminal epithelial cells surrounding the implanting embryo also undergo apoptosis prior to or during implantation in mice, rats and hamsters (Parr et al., 1987; Tassell et al., 2000; Correia-da-Silva et al., 2004; Zhang and Paria, 2006). The balance between cell proliferation and apoptosis is crucial for successful embryo implantation since increased levels of apoptosis have been reported to be associated with pregnancy failure in humans (Kokawa et al., 1998).

K⁺ channels are well documented for their role in cell proliferation and apoptosis in many cell types or under pathological conditions such as cancer (Jehle et al., 2011). Several K⁺ channels, including human eag-related gene (HERG), two-pore potassium (K2P) channels and the intermediate-conductance Ca²⁺-activated K⁺ channels, are found in human endometrial cancer cells, and pharmacological studies have suggested their roles in cell proliferation and cancer progression (Cherubini et al., 2000; Suzuki and Takimoto, 2004; Wang et al., 2007; Patel et al., 2013). Although these results are limited to cancer conditions, it should be noted that embryo implantation is considered an invasive and progressive process, similar to that of cancer, sharing common pathways and factors (Murray and Lessey, 1999; Stewart, 2007). The observed involvement of K⁺ channels in proliferation in endometrial cancer cells might also suggest the possible involvement of K⁺ channels in the process of embryo implantation. K⁺ channels are also reported to be involved in cell apoptosis (Jehle et al., 2011) in a number of cell types (Lang et al., 2004; Burg et al., 2008). Although no study has reported a similar role for K⁺ channels in endometrial cells, either epithelial or stromal, it is likely that these channels may also regulate apoptotic process prior to or during embryo implantation; however, this awaits further investigation.

In a recent study, the large-conductance calcium-activated potassium channel (BKCa) has been identified in human EECs with higher expression level at the mid-secretory compared with the proliferative phase of the menstrual cycle (Zhang et al., 2012). Interestingly, endometrial samples collected at mid-secretory phase from women undergoing IVF showed lower protein levels of BKCa in those with a failed pregnancy compared with those with a successful pregnancy (Zhang et al., 2012). Consistently, embryo implantation rate in mice was reduced after injection of siRNA targeting BKCa. These results suggest that BKCa may be important for embryo implantation. Although the study also showed that BKCa inhibition or knockdown caused reduction in the expression of implantation-associated genes in ISK cells, including Lif, Integrin-3, Claudin-4, and Dkk-1, how BKCa contributes to implantation and the underlying mechanisms have not been elucidated.

Our understanding of the role of endometrial K⁺ channels in implantation remains far from complete. In general, K⁺ channels are well known for their essential role in determining cell membrane potential (Armstrong and Hille, 1998; Ruan et al., 2008). It would be interesting to see whether endometrial K⁺ channels could modulate membrane potential and regulate VDCCs in the EECs leading to decidualization, as demonstrated for ENaC. Epithelial K⁺ channels, by operating with other epithelial ion transporters/channels, are known to play an important role in epithelial fluid transport in airways (Manzanares et al., 2011) and the kidney (Holtzclaw et al., 2011). A similar role of K⁺ channels in endometrial Cl⁻ secretion has been observed (Chan et al., 1997a; Fong et al., 1998); however, the exact molecular entity for the responsible K⁺ channels has not been identified. BKCa or other K⁺ channels may also be involved in the regulation of cell proliferation and apoptosis during embryo implantation; however, the underlying mechanisms are currently not known. Future effort should be made to enhance our understanding of the molecular mechanisms regulating these processes that are fundamental to the success of embryo implantation.

Others

In addition to the ion channels discussed above, a number of other ion channels have been demonstrated, or suggested, to be present in the endometrium. For example, Ca²⁺-dependent Cl⁻ channel (CaCC) has been functionally identified by Ussing chamer and patch-clamp experiments in mouse EECs, exhibiting sensitivity to extracellular ATP or UTP (Chan et al., 1997b, 2000; Wang and Chan, 2000). However, the molecular entity for this CaCC has not been identified and its exact role in implantation remains unclear.

In addition, ligand receptor ion channels have also been found in the endometrium and implicated in implantation. Purinergic receptor subtype X (P2X) is a family of ATP-activated nonselective cation channels including P2X(1–7) (North, 2002). P2X7 was found in rat endometrial epithelium and up-regulated at the time of implantation (Slater et al., 2002a, b), which has been taken to suggest its involvement in implantation, though no mechanism has been revealed.

γ-aminobutyric acid (GABA) receptor type A (GABA₅R) is a family of ligand-gated Cl⁻ channels (Sadeghi and Taylor, 2010). The subunit π of...
GABA<sub>A,R</sub> (GABA<sub>A</sub>-π) has been detected in human EECs and ESCs throughout the menstrual cycle but are significantly increased at the window of implantation. In ISK cells, GABA<sub>A,R</sub> appears to be subject to regulation by progesterone via the HOXA10-pathway (Fig. 2), which is known to be essential for endometrial receptivity and decidualization. However, precisely how GABA<sub>A,R</sub> is involved in the process awaits further investigation.

**Concluding remarks**

Studies reported over the last two decades have identified a number of ion channels in the endometrium of different species, including rodents and humans. While the functional roles of these ion channels in the endometrium are far from understood, some of these ion channels are emerging as key players in various processes of embryo implantation. Most endometrial ion channels are tightly regulated by ovarian hormones or factors derived from the implanting embryo, consistent with their possible involvement in implantation. As depicted in Fig. 2, some of these ion channels have been demonstrated or suggested to be involved in one or more of the following events pertinent to implantation: (i) regulation of uterine electrolyte and fluid transport, particularly by the interplay between CFTR and ENaC, together with K<sup>+</sup> channels and other transporters, to bring about the uterine luminal fluid reduction necessary for locking the embryo in place prior to implantation; (ii) promoting decidualization, such as ENaC by sensing signals, either mechanical or chemical, from the embryo and transducing them into downstream cellular responses leading to stromal decidualization; (iii) regulation of genes associated with endometrial receptivity and embryo implantation. It has also been suggested that ion channels, especially Ca<sup>2+</sup> channels, may play a role in regulating blastocyst adhesion to the endometrium, although the underlying mechanism has not been elucidated. The importance of endometrial ion channels in regulating implantation is highlighted by the observed implantation failure in animals or humans due to abnormal expression or function of some of the ion channels, exemplified by ENaC and BK<sub>Ca</sub>. With the advancement in our understanding of details of the involvement of ion channels in the course of implantation, mysteries surrounding implantation have begun to unfold. However, the roles of some of the endometrial ion channels, such as GABA receptor channels and P2X<sub>2</sub>, in implantation are yet to be elucidated. Of note, accumulating evidence has also indicated the importance of water channels (aquaporines—AQPs) in the process of embryo implantation (Huang et al., 2006). It is anticipated that the interplay between AQPs and other ion channels may be important in regulating uterine luminal fluid volume during implantation since protein—protein interaction between CFTR and AQP9 has been demonstrated in the male reproductive tract (Cheung et al., 2003; Pietermont et al., 2008). Further investigation along this line may reveal the delicate regulation of embryo implantation by the complex network of ion channels and water channels. While most of the current investigations are focused on the early events of implantation, future studies into the detailed involvement of endometrial ion channels in the full spectrum of the implantation process are expected to provide insights into the pathogenesis of some disorders/diseases occurring in the later course of pregnancy, such as placental insufficiency, pre-eclampsia and preterm birth. Advanced studies may also be designed to explore the potential of some of the ion channels as targets for the diagnosis and treatment of infertility or related diseases, as well as novel contraceptives.

**Authors’ roles**

H.C.C. conceived the idea and Y.C.R. designed the review. Y.C.R. and H.C. performed data acquisition and analysis. Y.C.R., H.C. drafted manuscript and H.C.C. revised and approved. Y.C.R. drew the figures.

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**Conflict of interest**

None declared.

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