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Review

Navigating the site for embryo implantation: Biomechanical and molecular regulation of intrauterine embryo distribution

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ABSTRACT

The distribution of intrauterine embryo implantation site(s) in most mammalian species shows remarkably constant patterns: in monotocous species such as humans, an embryo tends to implant in the uterine fundus; in polytocous species such as rodents, embryos implant evenly along the uterine horns. These long-time evolved patterns bear great biological significance because disruption of these patterns can have adverse effects on pregnancies. However, lack of suitable models and in vivo monitoring techniques has impeded the progress in understanding the mechanisms of intrauterine embryo distribution. These obstacles are being overcome by genetically engineered mouse models and newly developed high-resolution ultrasound. It has been revealed that intrauterine embryo distribution involves multiple events including uterine sensing of an embryo, fine-tuned uterine peristaltic movements, time-controlled uterine fluid reabsorption and uterine luminal closure, as well as embryo orientation. Diverse molecular factors, such as steroid hormone signaling, lipid signaling, adrenergic signaling, developmental genes, ion/water channels, and potentially embryonic signaling are actively involved in intrauterine embryo distribution. This review covers the biomechanical and molecular aspects of intrauterine embryo distribution (embryo spacing at the longitudinal axis and embryo orientation at the vertical axis), as well as its pathophysiological roles in human reproductive medicine. Future progress requires multi-disciplinary research efforts that will integrate in vivo animal models, clinical cases, physiologically relevant in vitro models, and biomechanical/computational modeling. Understanding the mechanisms for intrauterine embryo distribution could potentially lead to development of therapeutics for treating related conditions in reproductive medicine.

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1. Introduction

Embryo implantation has been commonly considered as the first step towards embryo-maternal interaction and is critical for further embryo development (Chen et al., 2009a; Dey, 2010; Wang and Dey, 2006). In the past decades, there have been tremendous advances towards understanding the physiological and molecular events during embryo implantation, including the establishment of uterine receptivity, blastocyst activation, and embryo-uterine interactions (Dey et al., 2004; Lim and Wang, 2010; Wang and Dey, 2006). However, much less progresses have been made towards understanding the mechanisms that control intrauterine embryo distribution, which are equally important for successful embryo implantation (Chen et al., 2011b). The preimplantation embryo is about 0.1 mm in diameter and much smaller compared to the size of the uterine cavity, and thus, the precise spatiotemporal transport and orientation of such a small object within the uterine cavity apparently involves fine-tuned mechanisms that coordinate the floating embryo near the uterine wall. The optimal intrauterine embryo distribution should provide the blastocyst(s) with appropriate localization and orientation within the intra-uterine environment in order to establish and maintain a successful pregnancy.

In most studied mammalian species, the spatiotemporal intrauterine embryo distribution is highly consistent. For example, in polytocous species such as rabbit, pig, rat and mouse, the embryos enter the uterine horn at late morula or early blastocyst stage, followed by embryo implantation in an evenly distributed pattern along the longitudinal uterine axis (Fig. 1A), which is usually referred as "embryo spacing" (Perry and Rowlands, 1962; Anderson and Parker, 1976; Boving, 1956; Mossman, 1937; O'Grady and Heald, 1969; Restall and Bindon, 1971; Wimsatt, 1975). In addition, intrauterine embryo distribution in these species also includes the orientation of the implanting blastocyst at the antimesometrial pole of the uterine vertical axis, with inner cell mass (ICM) positioned at the mesometrial side of the implantation chamber (Alden, 1945; Boving, 1972; Mossman, 1937; Wimsatt, 1975) (Fig. 2A). In monotocous species such as monkey, baboon and human, the implantation site is normally found at the uterine fundus (Bulletti and De, 2006; Fanchin and Ayoubi, 2009; Hafez, 1980; Heuser and Streeter, 1941) (Fig. 3B and C), with the ICM facing the uterine attachment site (Boving, 1959; Heuser and Streeter, 1941). From a developmental and physiological aspect, embryo spacing along longitudinal axis prevents embryo overcrowding and precludes embryonic loss due to unnecessary nutritional and space competition, while embryo orientation at the uterine vertical axis blueprints the position for subsequent embryonic axis and the formation of placenta.

The repeatable and programmed intrauterine embryo arrangement rather than a random distribution apparently implies rigid control mechanisms, which are probably evolved from long-time selection and adaptation to various parameters of a certain species, such as uterine shape, number of embryos, walking mode, local nutritional supply, etc. However, despite the interesting phenomenon of regulated intrauterine embryo distribution discovered for more than a century, there is still limited information for the underlying cellular and molecular mechanisms (Chen et al., 2009b; Dey, 2005). The facts that intrauterine embryo distribution is difficult to be monitored *in vivo* and could not be mimicked *in vitro* have likely contributed to the little progress in the field. Previous underestimation of its importance for ongoing gestation may have also led to inadequate research effort on this particular area.

In recent years, studies from genetic mouse models as well as clinical observations indicated that a fine-tuned regulation of intrauterine embryo location is critical for ongoing pregnancy because disruption of such process will cause abnormal

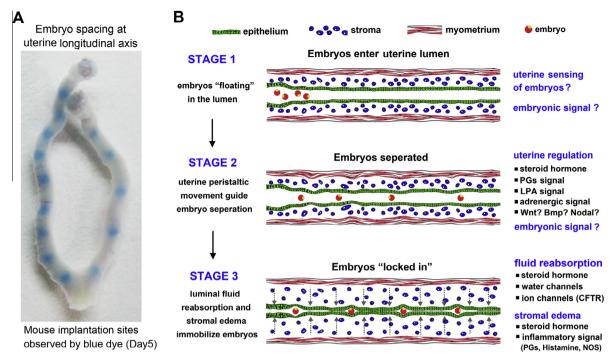


Fig. 1. Regulatory factors controlling embryo spacing along uterine longitudinal axis (A) Illustration of normal and abnormal embryo spacing in Day 6 pregnant mice (morning finding of vaginal plug as Day 1). 0.1 ml blue dye was injected intravenously 10 min before sacrifice of mice on Day 5 morning. The blue band showed an implantation site evenly spaced along uterine horn. (B) Schematic diagrams demonstrating the process and regulations of embryo distribution along the uterine longitudinal axis.

embryo development, miscarriage and other pregnancy complications such as placenta previa or cornual pregnancy (Fig. 3A–C) (Bulletti and De, 2006; Chen et al., 2011b; Lim and Wang, 2010; Song et al., 2002; Ye et al., 2005). High-resolution ultrasonic imaging, magnetic resonance imaging (MRI), physiologically relevant mathematical/computational models and laboratory simulations (Eytan et al., 2007a, 2001b, 1999; Eytan and Elad, 1999; Togashi, 2007; Yaniv et al., 2009), also enabled further observations into the detailed process leading to intrauterine embryo distribution. These new data have rekindled great interests in this old yet fascinating filed. Therefore, a thorough review of the mechanisms and clinical implications of intrauterine embryo distribution seems timely. This review will summarize existing knowledge from animal models (mostly from rats and mice) and clinical studies (with an emphasis on *in vitro* fertilization-embryo transfer (IVF-ET) practices) in order to promote the much needed progresses in the field and for exploring its therapeutic values.

2. Previous and current theories regarding intrauterine embryo distribution: the biomechanical nature

Regulated intrauterine embryo distribution in polytocous species was originally noticed over a century ago and became the interest of many early researchers. Embryo spacing along the longitudinal axis is macroscopically visible at early stage through the decidual swelling, and could be visualized before decidualization at the time of embryo attachment via intravenous blue dye injection (Psychoyos, 1960) (Fig. 1A); while embryo location along uterine vertical axis at implantation (Fig. 2A) requires anatomical/histological approaches with careful observations. In pioneering studies with rabbit, it was described that the implanting embryos were invariably found at the uterine wall opposing the mesometrium and the embryonic area (ICM) was always oriented towards the mesometrial pole (Asslieton, 1894). Since the driving force of such constant embryo orientation was difficult to discuss at the time, Asslieton (1894) stated that "...This is a very important fact. It is probably necessary for the development of the rabbit that it should be thus situated. It would be very awkward if the embryo became fixed in any other position. The shape of the uterus at this stage, and the shape of the blastodermic vesicle that this stage are so beautifully adapted one to the other as to render any other position almost impossible..." (Asslieton, 1894). This intrauterine embryo distribution pattern in spacing and orientation was later found to be shared in rodents as well (Wimsatt, 1975). The following paragraphs review the theories about intrauterine embryo distribution at longitudinal and vertical axes.

2.1. Factors affecting embryo spacing along uterine longitudinal axis

An early hypothesis stated that after embryos entered the uterus, each implanting embryo might form a "refractory zone" around its location, thus preventing other embryos to attach close by. Under this model, Mossman (1937) proposed that the first embryo to be implant within the uterine horn was the closest to the uterine–oviduct junction, followed by those more

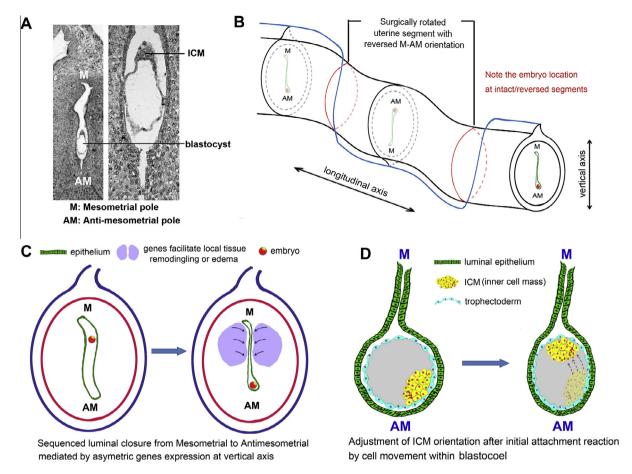


Fig. 2. Regulatory factors involved in embryo orientation at uterine vertical axis (A) Demonstrative pictures of mouse embryo localization along the M-AM axis at Day 5 implantation site. The blastocyst implantation happens in the anti-mesometrial pole of uterine lumen (left). Note the orientation of ICM is towards the uterine mesometrial pole (right). ICM, Inner Cell Mass (Reprint from Chen et al. (2009a)). (B) Illustrative pictures showing antimesometrial localization of embryo at intact and surgical reversed uterine segment in rat. Picture adapted from Alden (1945). (C) A hypothetical model showing sequenced luminal closure from mesometrial to anti mesometrial regulated by asymmetrical gene(s) expression. (D) A hypothetical model showing adjustment of ICM orientation after trophectoderm attached to luminal epithelium.

and more remote from the junction, which resulted in the pattern of embryo spacing (Mossman, 1937). However, this hypothesis could not explain how the regulation was made to estimate the number of embryos in the uterus, and how were the distances between the first and successive embryos controlled in order to yield the evenly spaced pattern within the uterine horn. Actually, when extra embryos (compared with normal pregnancy) were transferred into a pseudopregnant uterine horn, multiple implantation sites could be very closely apposed, suggesting that the "refractory zone" might not exist and each uterine segment have the potential for embryo implantation. A later study with mice by McLaren and Michie (1959) were unable to confirm Mossman's claim and discounted his hypothesis. They suggested that "the only mechanism for spacing implantations in the uterine horn may be the simple stirring brought about by uterine movements" (Mclaren and Michie, 1959).

2.1.1. Uterine peristaltic movements

The time course of embryo spacing in mice has been revealed by serial longitudinal sections at different time points before implantation. After the embryos entered the uterine horn in the morning of gestation Day 4 (implantation initiates around midnight of Day 4 in mice) they tended to group together in the uterine horn. Then, they gradually separated along the longitudinal axis before their positions were finally fixed (Restall and Bindon, 1971). These observations further suggested that the final embryo transportation might be mediated by uterine mechanical forces. A series of works using *in vivo* video-laparoscopy technique have recorded distinct myometrial contractile activities in rat during oestrous cycle, post-copulation and preimplantation periods (Crane and Martin, 1991a,b,c). The different uterine peristaltic patterns observed at each period have been suggested to be responsible for different physiological functions, such as transport of sperm and embryo. Interference of myometrial contractile activities using pharmaceutical approaches such as relaxin, adrenergic drugs and prostaglandin synthesis inhibitor (indomethacin) could disrupt longitudinal embryo spacing, as well as embryo

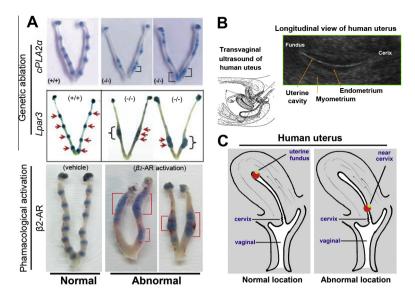


Fig. 3. Abnormal intrauterine distribution in mice and human (A) disrupted embryo spacing in genetic mutant mice or pharmacological treatment at preimplantation (Reprint from Song et al., 2002; Ye et al., 2005; Chen et al., 2011b) (B) Longitudinal view of uterus during transvaginal ultrasound (C) Illustrative pictures showing normal and abnormal embryo implantation locations in human pregnancy. The abnormal implantation location will further lead to placenta previa.

orientation at uterine vertical axis (Chen et al., 2011b; Kennedy, 1977; Kinoshita et al., 1985; Legrand et al., 1989, 1987; Pusey et al., 1980; Rogers et al., 1983; Wellstead et al., 1989). Uneven embryo spacing has been observed in genetically engineered mouse models, such as $Pla2g4\alpha^{(-)}$ mice deficient for cytosolic phospholipase $A_{2\alpha}$ and $Lpar3^{(-)}$ mice deficient for the third receptor for lysophosphatidic acid (LPA) (Song et al., 2002; Ye et al., 2005). Both mutant mice showed decreased uterine prostaglandins, which is essential for proper myometrium contractility. Indeed, it has been demonstrated that $Lpar3^{(-)}$ uterus lacks LPA_3 agonist-stimulated uterine contractile response(Hama et al., 2007). All these evidences support the consensus that uterine contraction mediated peristaltic movements are essential for embryo spacing along the uterine longitudinal axis (Fig. 1B).

2.1.2. Intrauterine fluids and other potential factors

In addition to myometrial contraction, the intraluminal uterine fluids and the endometrium must also be taken into account for intrauterine embryo distribution. The endometrium, especially the intraluminal uterine luminal epithelium, may detect the presence and real-time location of the embryos within the lumen (by physical or chemical signals from embryos) (Fig. 1B). The intraluminal uterine fluid buffers and carries the embryos before they implant into the uterine wall. When the embryos have been appropriately located along the uterine horn, the reabsorption of intra-luminal uterine fluid may play an important role in luminal closure to "lock" the embryos on the right locations (Eytan et al., 2001c, 2004; Yaniv et al., 2003) (Fig. 1B). After the embryo is physically apposed with the luminal epithelium, the trophoblast–endometrium attachment reaction will finally determine the intrauterine location of embryo implantation, which involves active regulation of adhesion molecules including integrins, selectins, glycoproteins, as well as other paracrine and juxtacrine systems such as HB-EGF signaling. The importance of these molecules has been comprehensively summarized in previous reviews (Armant, 2005; Carson et al., 2000; Norwitz et al., 2001; Red-Horse et al., 2004; Wang and Dey, 2006). In addition, post-implantation mechanisms such as differential growth of the myometrium are also taken into consideration to further reinforce the evenly spaced pattern in polytocous species (Finn, 1968).

Finally, although the concept of "refractory zone" surrounding each embryo has been discarded, there are still possibilities that the process of embryo spacing involves signal gradient formed by embryonic secretion or by region specific uterine secretions (Yoshinaga, 2010), which might involve the distribution of uterine glands (Hondo et al., 2007), and secretory proteins such as Sfrp2 (Secreted Frizzled-related protein 2) with inhibitory roles for implantation (Mohamed et al., 2005). Some of the above mentioned topics will be discussed more extensively in later sections.

2.2. Factors affecting embryo orientation along uterine vertical axis

In both monotocous and polytocous species the embryos have constant orientation along the uterine vertical axis at the time of implantation. Technically, the phrase "embryo orientation" at this time contains two levels of definitions (Wimsatt, 1975): positioning of the implanting embryo at the antimesometrial pole of the uterine vertical axis and the ICM location within the implanting blastocyst (Fig. 2A). This remarkably constant blastocyst and ICM orientations in the uterine vertical

axis are supposed to be critical for subsequent establishment of embryonic axis and embryo development, since disruption of these orientations is associated with early embryonic death (Arman et al., 1998). To date, the intrinsic and extrinsic driving mechanisms that determine embryo orientation at the implantation sites remain unclear. Here we will discuss mechanical and biological regulations that control intrauterine embryo orientations.

2.2.1. Uterine factors dictate the antimesometrial location of implanting embryo

When the embryos entered the uterine lumen (i.e., morning of Day 4 in mice), they were found at random locations within the uterine cavity either at mesometrial, middle or antimesometrial region along the vertical axis (Restall and Bindon, 1971). However, by the time the embryos attached to the uterine wall, they were uniformly located towards the antimesometrial extremity of the uterine cavity, concurring with the embryo spacing process in the longitudinal axis (Wimsatt, 1975). There are strong evidences to suggest that the antimesometrial allocation of embryo is guided by uterine rather than embryonic factors. When beads, tissue blocks or air bubbles rather than embryos were transferred, they all invariably end up locating at the antimesometrial pole similar to normal blastocyst (Paria et al., 2001; Beer and Billingham, 1970; Hetherington, 1968; McLaren, 1969; Wilson, 1960), which indicate that the antimesometrial localization does not require the presence of living embryos. As for the regulating factors responsible for this constant observation, it has been initially postulated that the antimesometrial location of the embryo might be regulated by gravity forces (Burckhard, 1901). Subsequent experimental work has, however, disproved this hypothesis: it was found that the implanting blastocysts were also located at the antimesometrial pole in surgically inverted rat uterine segment (Fig. 2B) (Alden, 1945). This experiment suggested that antimesometrial location was programmed by intrinsic factors of the uterus (Alden, 1945). However, a recent study showed that keeping mice in a supine position from the pre-implantation to implantation period could lead to embryo location at the mesometrial site of the uterine lumen. The authors thus suggested an involvement of gravity on intrauterine embryo location (Sugiyama et al., 2010). However, the authors did not show the embryo location at the time of attachment when the embryo localization was fixed (Sugiyama et al., 2010), which makes the conclusion contestable. On the other hand, the unusual body position described in the work would cause considerable body stress, which could disrupt normal uterine function thus embryo location (Chen et al., 2011b; Polidoro et al., 1973; Wang et al., 2004a). Therefore, current consensus remains that the antimesometrial disposition of the embryo is primarily programmed by intrinsic factors of the preimplantation uterus, while the effects of external forces such as earth gravity and body movements should be minimal in affecting the process. In support to this idea, clinical observations showed that the routine use of bed rest (as to limit body activity) didn't not show obvious improvement to pregnancy outcome in women undergoing ET in IVF cycles (Li et al., 2011).

The preimplantation hormonal environment is considered to be the master regulator in deciding the antimesometrial embryo localization. In experimentally induced delayed embryo implantation mouse model, in which the ovaries were removed in the morning of Day 4 followed by daily progesterone injection, the blastocysts were dormant without initiating attachment reaction with the luminal epithelium, yet they were constantly found at the antimesometrial pole. A single injection of small amount (10 ng) of 17β-estradiol (E2) could then activate the dormant blastocysts to initiate implantation (Finn and Martin, 1974). These observations demonstrated that while E2 is essential for implantation initiation, progesterone was critical for antimesometrial embryo positioning. The exact cellular and molecular events leading to antimesometrial embryo location remain unclear. One hypothesis could be that antimesometrial embryo positioning might involve sequential luminal closure from the mesometrial to the antimesometrial pole, thus pushing the embryo towards the antimesometrial extremity (Fig. 2C). If this hypothesis is correct, a serial tissue remodeling or edema from antimesometrial to mesometrial extremity in the stroma bed might be responsible for facilitating the sequenced luminal closure, the apical surface of epithelium might also make correspondent changes as to seal the lumen. In this regard, signaling molecules/genes with asymmetrical distribution/expression along the mesometrial-antimesometrial axis (dorsal-ventral axis) during preimplantation might be involved in this process. Indeed, it has been reported that prostaglandin E2 (PGE2) receptor EP3 is expressed in a subpopulation of cells in the stromal bed at the mesometrial side in gestation Day 4 pregnant or pseudopregnant mouse uteri and quickly disappears after the initiation of embryo attachment (Yang et al., 1997). This dynamic spatiotemporal expression pattern might suggest that EP3-mediated PGE2 signaling could possibly be involved in the proposed sequential luminal closure at uterine vertical axis. However, the EP3 knockout mice didn't show obvious defects in embryo orientation. This observation suggests that EP3-mediated PGE2 signaling is either not critical for embryo positioning or more possibly, the deletion of EP3 could be functionally compensated by other PGE2 receptors for PGE2 signaling, or compensated by other genes with gradient expression along the mesometrial-antimesometrial axis, such as fibroblast growth factors 10 (fgf10), noggin, etc. at the time of embryo distribution (Paria et al., 2001; Wang and Dey, 2006). Interestingly, a recent study by uterine deletion of MSX Homeobox Gene Msx1, a gene robustly expressed in epithelial cells of mouse uteri on Day 4 pregnancy, resulted in abnormal implantation and subsequent pregnancy loss (Daikoku et al., 2011). The abnormality of embryo implantation in the mutant mice includes abnormal embryo spacing at longitudinal axis; as well abnormal embryo location at the vertical axis, in which case the implanting embryo was found at the middle of mesometrial-antimesometrial axis (Daikoku et al., 2011). These observations might involve abnormal epithelial polarization (Daikoku et al., 2011). The genes critical for embryo positioning at antimesometrial locations remain to be further identified.

2.2.2. The intrinsic pulse and extrinsic guides for ICM orientation

The orientation of ICM by the time of blastocyst implantation is another largely unexplored area. It was reported that by the time the early attachment reaction first occurs, the ICM directions were randomly positioned at the mesometrial,

antimesometrial pole or the lateral sides (Kirby et al., 1967). We had similar observation from cross-sections of the early stage implantation sites at Day 4 24:00. However, a few hours later, ICM was constantly found at mesometrial pole in the morning of Day 5, suggesting that ICM orientation was established during the initial hours of embryo implantation. One hypothesis is that by the time of initial attachment, when the trophectoderm has become physically immobilized to the uterine epithelium, the adjustment of ICM orientation might result from ICM cell migration (Fig. 2D). Electron-microscopic studies have provided evidences that by the time the ICM adjusts its position within the blastocoel, the cell junction between the ICM and the trophoblast shell is loose, there is considerable intercellular space within the ICM, and there are no desmosomes uniting the cells (Kirby et al., 1967). These morphological observations support the notion that the cells within ICM are not tightly attached to each other and to the trophectoderm layer, therefore are capable of migrating inside the blastocoel (Kirby et al., 1967). Indeed, recent studies using time-lapse cell tracking system has revealed dynamic cell sorting in the ICM at blastocyst stage (Meilhac et al., 2009; Morris et al., 2010), supporting the notion that the ICM cells at blastocyst stage are highly mobile within the blastocyst. However, there are also possibilities that the embryo rotate as a whole and the ICM stay static to the trophectoderm where they initially contacted.

Regarding the molecules regulation of embryo orientation, it has been reported that Fgfr2 is asymmetrically expressed in the mouse blastocyst with low levels in the polar trophectoderm and high levels in the mural trophectoderm (Haffner-Krausz et al., 1999). Fgfr2 $^{-/-}$ embryos are lethal at pre-gastrulation due to incomplete decidualization and disrupted blastocyst orientation in the implantation chamber (Arman et al., 1998). These observations suggest that the asymmetric expression of fgfr2 along the embryonic-abembryonic axis might facilitate the correct orientation of the implanting blastocyst (Haffner-Krausz et al., 1999). It will be interesting to identify other genes with similar expression pattern as fgfr2 in the blastocyst and determine their functions in blastocyst orientation.

The involvement of fgfr2 in blastocyst orientation as well as successful in vitro culture of postimplantation embryo to go beyond gastrulation and even reach somite stage (Hsu, 1971, 1972; Morris et al., 2012; Pienkowski et al., 1974; Wilson and Jenkinson, 1974), suggested the involvement of intrinsic embryonic signals in embryo orientation and development. However, these observations do not rule out the potential involvement of uterine factor(s) in this process. For example, FGF may derive from the uterus to activate FGFR2 in the embryo (Samathanam et al., 1998). And it should be noticed that the success rate of in vitro embryo development beyond gastrulation stage is low, the overall shape and embryonic axis developed in vitro is also not identical to that observed in vivo (Wilson and Jenkinson, 1974). Even in in vitro blastocyst and endometrium co-culture system, the blastocyst attachment sites and the embryo orientation are different from that observed in vivo (Tan et al., 2005b). These observations imply that the uterine environment is at least involved in optimizing the establishment of embryonic axis, possibly by influencing ICM orientation at implantation (Mesnard et al., 2004; Weber et al., 1999). Indeed, given the interactive nature of embryo-uterine cross-talk at implantation, it is a reasonable assumption that the paracellular communication between luminal epithelium and blastocyst, as well as the physical shape of the implantation chamber might both provide critical information for correct ICM orientation around the time of implantation. Beyond the implantation stage, the embryo orientation within uterine might also require regulated decidualization process, possibly guided by region-specific expression of developmental genes (Paria et al., 2001; Wang and Dev, 2006) that lead to patterned uterine shape and differential tissue remodeling at decidualization. The regulated uterine decidualization process might be actively involved in finely adjustment of correct embryo orientation at egg cylinder and gastrulation stage. It is still technically challenging to develop non-invasive approaches for monitoring ICM orientation process in vivo.

3. Molecular signaling for intrauterine embryo distribution

Diverse molecules and signaling pathways involved in intrauterine embryo distribution have been identified using physiological, pharmacological, and genetic approaches. However, the precise details and hierarchical relationships for many of these factors are still far from clear. The following sections will focus on selected critical molecules and signaling pathways that fall into two major functional categories: (1) factors controlling uterine contraction that guide embryo migration. (2) The uterine locking system that immobilizes the embryos before attachment reaction.

3.1. Steroid hormones

In mammals, the implantation process requires precisely regulated ovarian hormones (Dey et al., 2004; Finn and Martin, 1974). In mice and rats, the hormonal environment in preimplantation uteri is progesterone primed, followed by a small surge of estrogen in the morning of Day 4 to prepare a receptive uterus for embryo implantation (Dey et al., 2004). The precise level of E2 at this stage is critical because abnormally hypo or hyper E2 level will both adversely affect uterine receptivity and decidualization (Curtis et al., 2002; Das et al., 2009; Finn et al., 1992; Ma et al., 2003; Milligan et al., 1995). Given the critical roles of progesterone (P4) and E2 interaction at this stage for embryo implantation, the proper interaction of P4 and E2 would also be responsible for the accurate intrauterine embryo distribution. It was reported that exogenous estrogen administration in rabbit while the embryos were in the uterus caused abnormal spacing of implantation sites, demonstrating that an imbalanced steroid hormones before implantation disrupted normal embryo spacing (Greenwald, 1957). Interestingly, another study in rat also found elevated E2 level and delayed/decreased P4 level were associated with abnormal embryo spacing and aggravated the effect of nicotine on disrupting embryo spacing (Yoshinaga et al., 1979). We also observed

that a single exogenous estrogen injection (150 ng/mice) before embryo attachment (at Day 4 pregnancy) could effectively disrupt embryo spacing in mice (unpublished). These data clearly indicated that the ratio of P4 and E2, especially the levels of E2 at preimplantation are critical not only for the establishment of uterine receptivity, but also for proper intrauterine embryo distribution.

In human, there is a striking difference in the concentrations of estradiol and progesterone within the utero-ovarian veins of the ipsilateral as compared to the contralateral side of the ovulated varies, which is thought to significantly influence directed sperm transport, as well as the selection of embryo implantation site (Kunz et al., 2000). These observations demonstrated that the local hormonal environments could substantially influence the course of intrauterine transport; and it is reasonable to imagine that disturbed hormonal environment before embryo implantation, either by habitual use of tobacco, or by hyperovarian stimulation during *in vitro* fertilization (IVF) cycle could adversely affect intrauterine embryo location.

The mechanisms by which P4 and E2 coordinately regulate intrauterine embryo distribution are complicated, but at least two major aspects are actively involved by: (1) affecting myometrial contractility, and (2) regulating intrauterine fluid homeostasis, which will be discussed below. We will also discuss the possible roles of embryonic estrogen in regulating intrauterine embryo migration and implantation.

3.1.1. Steroid hormones and uterine contractility

It has long been recognized that myometrial activity is finely regulated through interactions of E2 and P4 in rodents (Downing et al., 1981). The hormonal influence on uterine contractility has also been studied in human non-pregnant uteri and during IVF cycle before embryo transfer (Fanchin et al., 2000; Fanchin et al., 2001b; Oike et al., 1990). It is now established that the synergistic and counteractive effects of P4 and E2 are the primary regulators that govern the peristaltic pattern of uterine movements. By using ex-vivo uterine model, it is confirmed that the primary role of E2 is stimulatory on the contractile activity of myometrium, while that of P4 is mainly relaxant (Bulletti et al., 1993). In vivo video-laparoscopy in rats demonstrated that there were distinct myometrial contractile activities during oestrous cycle, post-copulation and preimplantation (Crane and Martin, 1991a,b,c). Non-invasive ultrasound imaging revealed three types of uterine peristaltic contractions in human uterus: cervico-fundal, fundo-cervical and isthmical peristaltic activity, which changes during the menstrual cycle and is controlled by the systemic and local steroid hormonal environments. (Fanchin et al., 2000; Kunz and Leyendecker, 2002). These data demonstrated the hormonal control of uterine peristaltic patterns, which would be responsible for different uterine functions such as sperm transportation, embryo transport and embryo implantation. Clinically, abnormal uterine contractility at the time of embryo implantation will cause sub-optimal intrauterine embryo location or event "push" the embryo out of the uterus, causing pregnancy complications such as placenta praevia or infertility. Therefore, determination of the optimal steroid hormone regimen for optimal uterine contractility during early pregnancy may be required to prevent abnormal uterine contractility, and worth future exploration.

The downstream signaling mediating the effects of steroid hormones on myometrial activity is an interesting topic that warrants further investigation (Aguilar and Mitchell, 2010). Several well-studied pathways in close relationship with intrauterine embryo distribution will be discussed in detail in later sections.

3.1.2. Steroid hormones and intrauterine fluid homeostasis

It is known that P4 and E2 ratio is the key determinant of intrauterine fluid homeostasis (Clemetson et al., 1977; Salleh et al., 2005). It was demonstrated in ovariectomized rats that E2 promoted secretion of sodium, potassium and water into the intrauterine lumen and P4 reabsorbed these substances (Clemetson et al., 1977; Salleh et al., 2005). It is an important but previously less considered fact that the fluid dynamics in the uterine lumen during preimplantation are closely associated with the course of intrauterine embryo distribution and implantation: after an embryo enters the uterine lumen, the intrauterine fluids presumably could act as a carrier buffer to protect and transport the floating embryo in concert with the uterine peristaltic movements. When the embryo has been transported to an optimal intrauterine location, the uterine luminal fluid must be reabsorbed timely to help "lock" the embryo from floating away, thus facilitates the interactions between the embryo and the uterine luminal epithelium for implantation initiation (Naftalin et al., 2002; Salleh et al., 2005). This assumption is supported by the observation that uterine luminal fluid reabsorption peaks at the expected time of implantation in rodents (Naftalin et al., 2002; Salleh et al., 2005).

The importance of uterine fluid control for embryo implantation is also well reflected in clinical protocols of embryo transfer (ET) after IVF, regarding the required fluid volume for successful ET into the uterine cavity. Limited volume of fluid (usually 20–60 µL) is co-transferred with embryo(s) into the uterine cavity to preclude the extensive floating of the transferred embryo(s) (Magli et al., 2008; Schoolcraft et al., 2001). Nevertheless, the transferred embryos could still "float" to certain distance because they do not always implant at the transferred site (Baba et al., 2000; Soares et al., 2008), Excessive uterine fluid at the time of implantation could lead to infertility. For example, hydrosalpinx (with blocked and fluid filled fallopian tube) is a common female infertility cause (Savaris and Giudice, 2007), and the leakage of hydrosalpinx fluid into the endometrial cavity could be a major cause of low IVF success rate in patients with hydrosalpinx (Chien et al., 2002; Hinckley and Milki, 2003; Savaris and Giudice, 2007; Strandell et al., 2001). Besides the potentially harmful nature of the hydrosalpinx fluid (which is disputable) (Strandell, 2000; Strandell and Lindhard, 2002), the most accepted explanation for the adverse effect of hydrosalpinx on failed embryo implantation after ET is that the excessive fluid disrupts the normal interactions between embryo and endometrium, leading to "flushing out" of the embryo or "floating-off" from an optimal implantation site that could lead to compromised embryo development or pregnancy complications such as placenta previa

(Eytan et al., 2001a,c; Hinckley and Milki, 2003; Romundstad et al., 2006; Strandell and Lindhard, 2002). These facts have highlighted the importance of uterine fluid, regardless of its origin, for intrauterine embryo distribution and implantation. Uterine water channels and ion channels are involved in regulating uterine fluid under the control of steroid hormones. They will be discussed in more details in later sections.

3.2. Prostaglandin and lysophospholipid signaling

Prostaglandin (PG) signal is one of the most extensively studied signal pathways that influence embryo implantation and embryo spacing. Preimplantation administration of indomethacin, an inhibitor of prostaglandin synthesis by targeting cyclooxygenases COX-1 and COX-2 (the rate limiting enzymes for PG synthesis), could delay embryo implantation and disrupt embryo spacing in rat without inhibiting ovarian steroidogenesis (Kennedy, 1977; Kinoshita et al., 1985; Wellstead et al., 1989). It was assumed that the PGs increased the vascular permeability at implantation sites and affected myometrial contractility (Dey et al., 2004; Shah and Catt, 2005). Estrogen could regulate uterine PG synthesis via the related enzymes (Chakraborty et al., 1996; Ham et al., 1975). Progesterone/estrogen ratio is important for myometrial PG synthesis and metabolism, also influences myometrial sensitivity to PGs via regulating PG receptors, which can mediate myometrial contraction or relaxation (Myatt and Lye, 2004). The essential role of PGs in embryo implantation and embryo spacing has also been demonstrated in gene knockout mouse models. Mice deficient of COX-2, the rate limiting for PG synthesis, have different degrees of implantation failure depending on the mouse genetic backgrounds (Lim et al., 1997; Wang et al., 2004b). Mice deficient of cPLA2α, which is involved in producing arachidonic acid (AA) for PG synthesis, have deferred implantation and disrupted embryo spacing (Song et al., 2002). Mice deficient of the third lysophosphatidic acid (LPA) receptor, lpa3, also have delayed embryo implantation and abnormal embryo spacing (Ye et al., 2005). Interestingly, reduced uterine PG production associated with decreased uterine COX-2 expression was detected in both cPLA2α knockout uterus and lpa3 knockout uterus (Ye et al., 2005). The above observations support the essential role of PG signaling in embryo implantation and spacing (Dey, 2005: Shah and Catt. 2005). In addition, LPA has stimulatory effect on isolated uterine smooth muscles (Tokumura et al., 1980), and LPA3 agonist-induced uterine contraction is abolished in lpa3-deficient uterus, which has embryo crowding (Hama et al., 2007). These observations support the involvement of LPA/PG mediated myometrial activity in embryo spacing.

Further studies from *lpa3*-deficient have demonstrated that embryo spacing and embryo implantation are two segregated events and different PGs play different roles in these two events. In *cPLA2* and *lpa3* knockout mice, exogenous PGE2 plus cPGI (the stable analogue of PGI2) could rescue implantation timing but not embryo spacing (Song et al., 2002; Ye et al., 2005). In addition, single embryo transferred into *lpa3* knockout pseudopregnant females, which did not pose embryo crowding, there was still delay in implantation, indicating that the delayed implantation timing is not resulted from embryo crowding in *lpa3* knockout mice (Hama et al., 2007). These data demonstrated that embryo spacing and embryo implantation timing are two differentially regulated events (Hama et al., 2007). COX-derived PGs include PGD₂, PGE₂, PGF_{2α}, PGI₂, and thromboxane A₂ (TxA₂), which could activate their respective GPCRs, DP₁₋₂, EP₁₋₄, FP, IP, and TP (Cha et al., 2006; Myatt and Lye, 2004). These PGs receptors are expressed in the uterus and mediate different effects in myometrial activity (Myatt and Lye, 2004; Yang et al., 1997). EP₁, EP₃, FP, and TP have contractile effects on the myometrium, while DP₁₋₂, EP₂, EP₄, and IP have relaxant effects (Bos et al., 2004; Cha et al., 2006; Myatt and Lye, 2004; Wang and Dey, 2005) (Fig. 4).

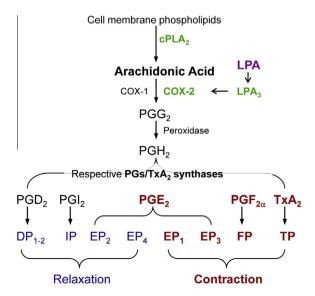


Fig. 4. Involvements of prostaglandin (PG) and Lysophospholipid (LPA) signaling in myometrial contractility and relaxation. COX-derived prostanoid signaling in myometrial relaxation and contraction as well as the hypothetical connections among cPLA2, LPA3, and COX-derived prostanoid signaling.

We hypothesized that COX-derived prostanoid(s) that could induce a contractile effect rather than a relaxant effect on the myometrium may alleviate embryo crowding since (1) PGE2 and cPGI can restore normal implantation timing but fail to rescue embryo spacing defect in $lpa3^{(-)}$ females and $Pla2g4a^{(-)}$ females; (2) PGE₂ can induce both contractile (via EP₁ and EP₃) and relaxant (via EP₂ and EP₄) effects, and cPGI only induces relaxant (via IP) effects; and (3) embryo spacing is thought to be achieved through uterine myometrial contractions(Legrand et al., 1989; O'Grady and Heald, 1969; Pusey et al., 1980). Among the GPCRs that mediate the signaling of COX-derived prostanoids, FP and TP exclusively mediate uterine contractility (Myatt and Lye, 2004). We demonstrated that a TP agonist, 11-deoxy $PGF_{2\alpha}$ (a synthetic analog of $PGF_{2\alpha}$), could partially alleviate the embryo crowding and partially restore on-time embryo implantation in the $lpa3^{(-)}$ females, whereas an FP agonist, fluprostenol (a metabolically stable analog of $PGF_{2\alpha}$), prevented embryo implantation regardless of genotypes, most likely due to its luteolytic effect (Ye et al., 2011). These results further demonstrate that PG signaling is involved in both embryo spacing and embryo implantation timing and PGs with a contractile effect on myometrium may have an important role in embryo spacing. Interestingly, the known upstream molecules regulating PG production, such as cPLA2α, LPA₃, COX-1 and COX-2, are all mainly or exclusively expressed in the epithelium of preimplantation uterus but not the myometrium (Chakraborty et al., 1996; Song et al., 2002; Ye et al., 2005, 2011), which is a major player in uterine contraction. It is likely that paracrine mechanism could be involved in PG signaling on embryo spacing and embryo implantation. However, several questions remain to be answered: (1) what uterine cell type(s) produces different PGs? (2) How do the PGs reach their respective receptors to exert their functions? (3) How does the contraction of myometrium finely control the embryo distribution within the uterine lumen, which is separated from the myometrium by the endometrium?

Besides the signal events mediated by prostaglandin and LPA receptors, it is possible that they might also interact with other regulators that control muscular contractility (Ye and Chun, 2010). For example, prostaglandin signaling has been linking with adrenergic signaling in several systems (McGraw et al., 2006), and it has been reported that some prostaglandin receptors were present on adrenergic neurons of the porcine uterine longitudinal muscle (Cao et al., 2008). LPA signaling has also been reported to regulate adrenergic receptor trafficking (Shumay et al., 2007). In the following section, we'll further discuss the role of adrenergic signaling in myometrium function regarding intrauterine embryo distribution.

3.3. Adrenergic signaling

The mammalian uterus is an organ with extensive sympathetic innervations, especially in the myometrial compartments (Chavez-Genaro et al., 2006; Houdeau et al., 1998; Latini et al., 2008). Noradrenalin release from sympathetic activation could act directly through uterine adrenergic receptors, which are regulated by ovarian steroid hormones (Engstrom et al., 2001; Hartley et al., 1983; Roberts et al., 1989). It has been established that noradrenalin-mediated myometrial contraction is critical for uterine functions such as intrauterine embryo distribution during early pregnancy (Chen et al., 2011b; Legrand et al., 1989, 1987) and initiation and progression of parturition during late pregnancy (Engstrom, 2003; Legrand and Maltier, 1986). Increased noradrenalin concentration in rat myometrium before implantation (Legrand et al., 1987) suggests potential roles of noradrenalin in implantation. Indeed, interfering adrenergic signaling at preimplantation by administration of α1-adrenoceptor antagonists could disrupt normal embryo spacing in rats (Legrand et al., 1987), which was believed to be a result of disregulated myometrial function, similar to the interference by relaxin, a potent myometrial contraction inhibitor (Rogers et al., 1983). Since myometrium has both alpha and beta-adrenergic receptors, which are responsible for contractility and quiescence of myometrium, respectively, the spatiotemporal distribution of adrenoceptor subtypes in the uterus could be a major modulator for myometrium tone throughout pregnancy (Bottari et al., 1983; Mesiano, 2004; Roberts et al., 1989), \(\beta^2\)-Adrenoceptor is mainly expressed in the myometrium with weak expression in the luminal epithelium of mouse preimplantation uterus. An abnormal transient activation of β2-adrenoceptor before implantation did not affect implantation timing but abolished normal uterine contractility and disrupted intrauterine embryo distribution, leading to regarded embryo development or embryo loss at mid-gestation in mice (Chen et al., 2011b). This study reveals the essential role of intrauterine embryo distribution on pregnancy outcome and supports the previous observations that embryo spacing and embryo implantation timing are segregated events (Hama et al., 2007; Ye, 2008; Ye et al., 2012; Ye and Chun, 2010). The abnormal intrauterine embryo distribution by β2-adrenoceptor agonist may involve not only embryo crowding at the longitudinal axis, but also possibly abnormal embryo orientation at uterine vertical axis, as previously described (Rogers et al., 1983).

Interestingly, we found that β 2-adrenoceptor agonist treatment could down-regulate Lpa3 mRNA expression in the preimplantation uterus (Day 4), which was specifically mediated through β 2-adrenoceptor (Chen et al., 2011b). These results suggest potential signaling interactions between these two G-protein-coupled receptors, β 2-adrenoceptor and LPA₃. Lpa3 mRNA is exclusively expressed in the uterine epithelium (strongly in the luminal epithelium and barely detectable in the glandular epithelium) of Day 4 pregnant mouse (Ye et al., 2005, 2011), whereas β 2-adrenoceptor is expressed in both uterine epithelium (weak, both luminal and glandular epithelium) and myometrium (strong) (Chen et al., 2011b). The potential signaling communications of these two GPRCs are possibly happen within the uterine epithelium, while paracrine signaling interactions could not be ruled out.

The clinical implications for abnormal adrenergic activation and pregnancy outcome are also noteworthy, because psychological/physical stress can elevate the concentrations of endogenous ligands for adrenoceptors, such as norepinephrine (Ferry et al., 1999). Epidemiological studies have shown that maternal stress at early pregnancy is strongly associated with various complications, such as bleeding and pregnancy loss during ongoing gestation (Ferry et al., 1999; Gold et al., 2007;

Neugebauer et al., 1996; O'Hare and Creed, 1995; Zubrick, 2008). Since a proper muscular contraction tone is important for normal implantation process (Bulletti and De, 2006; Fanchin and Ayoubi, 2009; Fanchin et al., 2001a), it is possible that aberrant uterine contraction in human pregnancy, either overactive or hypoactive, could result in embryo implantation at unfavorable sites (Fig. 3B and C), which is prone to miscarriage or other pregnancy complications, such as placenta praevia and cornual pregnancy (Chen et al., 2009a). Clinical observations also reveal that stressed women at implantation have abnormal patterns of uterine contraction and abnormal uterine peristalsis is directly linked with decreased pregnancy rate in human, which is possibly due to abnormal intrauterine embryo transport (Fanchin and Ayoubi, 2009; Fanchin et al., 1998; Kido et al., 2009; Robertson, 1939; Yoshino et al., 2010). Understanding adrenergic signaling in uterine movement could help explore the clinical values of adrenergic drugs in optimizing myometrial tone, especially during the time of embryo implantation.

3.4. Water channels and ion channels in intrauterine fluid homeostasis

The secretions of uterine luminal fluid at preimplantation provides a buffer for the pre-implanted embryo, while the reabsorption of uterine luminal fluid at the expected time of implantation helps "locking" the embryo and facilitates attachment reaction (Naftalin et al., 2002). Although the importance of luminal fluid secretion and reabsorption has long been recognized and the hormonal regulation of the volume and contents of uterine fluid has been established (Clemetson et al., 1977; Naftalin et al., 2002; Salleh et al., 2005), the underlying molecular mechanism for uterine luminal fluid dynamics is still an emerging topic. It has been suggested that uterine glands might play a primary role in the regulation of uterine fluid volume by switching from a secretory to an absorptive function under the appropriate endocrine control before implantation (Naftalin et al., 2002; Salleh et al., 2005). It was also suggested that the absorption of uterine fluid was mediated by transporting through the luminal epithelium (Clemetson et al., 1977), possibly involving irregular cytoplasmic projections ("pinopodes") on the apical side of luminal epithelium (Enders and Nelson, 1973; Nardo et al., 2002; Parr and Parr, 1974). Both of the above suggestions apparently involve ion and water transports. Therefore, steroid hormone-regulated water channels (aquaporins) and ion channels are the main candidates in the control of uterine fluid homeostasis.

Aquaporins (AQP) are a conserved family of transmembrane water channels widely distributed in various tissues and cell types (King et al., 2004). The AQP family members are divided according to their permeability characteristics. Classical AQPs are permeable to water alone (AQP1, 2, 4, 5). Some AQPs (AQP3, AQP7 and AQP9) are also permeable to other small molecules, such as glycerol and urea, and referred as aquaglyceroporines (King et al., 2004). Our recent study has suggested a potential role of AQP7 in mediating glycerol transportation as a potential energy substrate for the process of postimplantation decidualization (Peng et al., 2011). The function of AQPs in fluid homeostasis has long been suggested, however, the function of AQPs in uterine fluid regulation is unclear. AQP1 is the first water channel found in human endometrium (Li et al., 1994). AQP2 is localized in luminal and glandular epithelium of human endometrium in a cycle dependent manner, which closely correlated with serum ovarian steroid hormone (Feng et al., 2008; He et al., 2006; Hildenbrand et al., 2006). The spatiotemporal expression of AQPs in preimplantation rat uterus (Li et al., 1997; Lindsay and Murphy, 2004a,b, 2006, 2007) indicates that the redistribution of AOP5 before embryo implantation might contribute to the timely reabsorption of uterine fluid and might influence the antimesometrial location of the implanting embryo (Lindsay and Murphy, 2006). In preimplantation mouse uterus, AQP1 is localized in the myometrium and AQP5 is detected in the basal layer of glandular epithelium at the time of implantation and is sensitive to steroid hormone (Richard et al., 2003). Dynamic uterine AQP expression during periimplantation has also been detected in pig and dog (Aralla et al., 2009; Skowronski, 2010; Skowronski et al., 2009), The spatiotemporal expression patterns of AOPs are species-specific (Jablonski et al., 2003; Lindsay and Murphy, 2006, 2007; Richard et al., 2003). Several genome-wide arrays of mouse uteri reveal that AQP5 and AQP8 are sensitively upregulated by elevated estrogen level (Hewitt et al., 2005, 2003), correlating with increased uterine fluid upon estrogen treatment. AQP2, AQP5 and AQP8 are dynamically expressed in human endometrium in a cycle dependent manner (Jiang et al., 2010). These data indicate the hormonal regulation of AQPs and suggest potential roles of AQPs in uterine fluid regulation; however, a direct link is still missing. Although several AQPs knockout mice have shown reproductive phenotypes (Chen et al., 2011a; Sha et al., 2011; Su et al., 2010; Sun et al., 2009), none of them showed direct functional link regarding changed uterine fluid regulation, which suggested that under normal physiological conditions, AQPs may not be essential for uterine fluid regulation or more probably there is robust functional redundancy (Huang et al., 2006).

Uterine luminal fluid volume could also be regulated by ionic compositions, which change with the uterine environment (Casslen and Nilsson, 1984; Clemetson et al., 1970; Nordenvall et al., 1989). The cystic fibrosis transmembrane conductance regulator (CFTR), a CAMP-activated Cl channel, and epithelial Na⁺ channel (ENaC) have been proposed as the major ion channels in regulating uterine fluid secretion and absorption (Chan et al., 2002; Yang et al., 2004, 2003). Up-regulation of CFTR (either stimulation of hyper estrogen or by infection) at preimplantation is associated with abnormal uterine fluid accumulation and can adversely affect embryo implantation (He et al., 2010; Yang et al., 2011). Increased CFTR expression has also been observed in the human hydrosalpinx, a situation associated with excessive fluid accumulation in the fallopian tube (Ajonuma et al., 2002, 2005). These data suggest a role of CFTR in uterine fluid regulation and embryo implantation. ENaC has dynamic expression in the uterine epithelium. It may contribute to the ionic composition and water contents of uterine fluid. A recent study shows that abnormal upregulation of the Serum and Glucocorticoid-inducible Kinase (SGK1), a key regulator of sodium transport in mammalian epithelia, could cause abnormal uterine fluid handling and implantation failure in both human and mouse (Salker et al., 2011), which is associated with upregulation of ENaC in the luminal epithelium. This study highlighted the roles of ion channel in infertility cause.

It should be emphasized that besides the independent role of reported water and ion channels, all of them are under dynamic steroid hormone regulation, and the expression and activity of one channel could substantially influence the activity of other channels. For example, CFTR is a negative regulator of ENaC through direct interactions, with its absence enabling maximal ENaC activity (Berdiev et al., 2007; Gentzsch et al., 2010; Stutts et al., 1995). CFTR is also functionally related with the expression and functions of several aquaporin family members (Burghardt et al., 2003; Pietrement et al., 2008; Schreiber et al., 1999). The coordinated expression of these channels may provide an optimal uterine fluid/ionic environment for intrauterine embryo transport and intrauterine embryo localization, their potential function in intrauterine embryo distribution and implantation is an important area for future investigation.

3.5. Developmental genes

Many developmental genes are expressed in close relationship or functionally involved in the process of intrauterine embryo distribution. Wnt/ β -catenin signaling has a unique expression pattern in the preimplantation uterus. It is transiently activated in circular smooth muscle of early Day 4 pregnant but not pseudopregnant uterus. The sites of activation are evenly spaced pattern along the uterine horn (Mohamed et al., 2005). This expression pattern disappears in the muscular layer prior to the onset of blastocyst attachment. Instead, the expression of Wnt/ β -catenin signaling shifts to the implantation site (Mohamed et al., 2005). These findings raise the possibility that Wnt/ β -catenin signaling may play a role in directing normal embryo spacing in the mouse uterus (Carson, 2005).

This above study also showed that when embryos and sFRP2 were co-transferred in the proximal region of the uterine horn, the implantation sites occurred only in the distal region. This observation suggests that these embryos may have implanted in regions where sFRP2 protein concentrations were low (Mohamed et al., 2005). sFRP2 could significantly inhibit E2-induced uterine epithelial changes (Hou et al., 2004), suggesting that sFRP2-mediated signaling might act locally to influence the selection of optimal location for implantation. Another study demonstrated that co-transfer of blastocysts with beads soaked with BMP2 (but not BMP4) could induce abnormal embryo spacing in mouse (Paria et al., 2001). It was proposed that BMP2 in the uterine lumen might inhibit uterine contraction or induce abnormal luminal closure, leading to uneven embryo distribution alone the uterine horns (Paria et al., 2001). Bmp5/Nodal double mutant litters from double heterozygous crosses often contain two to four embryos of differing genotypes within the same deciduum, suggesting Bmp5/Nodal signaling may fuction in embryo spacing (Pfendler et al., 2000). However, the underlying mechanism is not fully understood. Using Nodal-lacZ transgenic mice, Nodal expression was revealed to be restricted to the glandular epithelium before embryo implantation, and there was a banding pattern at the inter-implantation along the uterine longitudinal axis, suggesting a role of Nodal in regulating the distribution of implantation sites (Park and Dufort, 2011). These data indicate that these genes critical for development may also play a role in embryo spacing.

3.6. Embryo emitted signal to direct uterine movement?

A blastocyst can secret various paracrine/autocrine factors. There have been implications and discussions on the influence of a blastocyst on uterine receptivity (Chen et al., 2009a; Matorras et al., 2005; Paria et al., 1993; Shiotani et al., 1993; Wakuda et al., 1999). There is a similar possibility that a blasocyst may emit signals to guide uterine changes (such as fine-tuned peristaltic movement) that are responsible for intrauterine embryo distribution (Fig. 1B). This concept is supported by the observations that (1) rat myometrial activity in pregnant and pseudopregnant uteri showed distinct patterns prior to implantation(Crane and Martin, 1991a); (2) the activation pattern of Wnt/β -catenin signaling in circular smooth muscle of early Day 4 pregnant uterus was absent from the pseudopregnant mouse uterus (Mohamed et al., 2005); and (3) blastocysts transferred into the preimplantation uteri could guide Nodal expression in the endometrium (Park and Dufort, 2011). However, the exact signal(s), being either the physical presence of the blastocyst and/or bioactive molecules secreted by the living embryo(s), has not been confirmed and/or identified.

Among the possible signals emitted by the blastocyst, estrogen is a candidate long-time thought of (Dickmann et al., 1977). Although preimplantation ovarian estrogen surge is critical for mice and rats to initiate attachment reaction, it is not required for some other mammalian species, such as golden hamster and guinea-pig (Dey et al., 2004; Finn and Martin, 1974; Wang et al., 2002). One hypothesis is that the blastocyst could secret estrogen to guide intrauterine embryo distribution and initiate implantation (Wang et al., 2002). This hypothesis is supported by the observation that the aromatase, which is responsible for estrogen conversion, is present in the hamster blastocyst (Reese et al., 2008), but not mouse blastocyst (Stromstedt et al., 1996). In human, there has not been enough evidence to support the requirement of estrogen for embryo implantation, and whether human blastocyst is a source of estrogen for intrauterine embryo distribution and implantation awaits further investigation.

Embryonic estrogen may be involved in intrauterine embryo distribution. It has been implicated that increased synthesis of estradiol by the porcine embryos occurred concomitantly with migration of the embryos and increased myometrial activity in porcine species (Pope et al., 1982a,b), and beads implemented with E2 migrated further and distributed in more evenly manner compared with beads implemented with cholesterol in porcine and sheep uteri(Nephew et al., 1992; Pope et al., 1982a). However, the later observation was not repeatable because later study showed that porcine uterine horn could not discriminate between E2 and cholesterol-releasing beads and lacks a coordinated ability to displace adjacent beads (Pope et al., 1986). The role of embryonic estrogen in guiding intrauterine embryo distribution awaits further clarification.

In addition to embryonic estrogen, embryonic PG signaling could be another factor influencing intrauterine embryo distribution. Prostaglandins were detected in rabbit blastocysts (Dey et al., 1980; Dickmann and Spilman, 1975). Enzymes for PG synthesis were detected in mouse blastocyst and with dynamic changes associated with blastocyst activation (Paria et al., 1998; Tan et al., 2005a). One hypothesis is that the embryo-derived PGs might activate uterine PGs receptors to regulate embryo spacing and embryo implantation. Also, another interesting studies has showed that AM(+/-) female mice heterozygous for adrenomedullin, a multifunctional peptide vasodilator, showed abnormal embryo spacing at the time of implantation, and the cause might be associated with decreased secretion of adrenomedullin from both maternal and embryonic sides(Li et al., 2006). If embryonic factors are indeed involved in intrauterine embryo distribution, how the embryo signal is sensed by the uterine luminal epithelium and transmitted to myometrium to guide muscular movement in real-time remains to be explored.

4. Emerging biomechanical/computational models to study intrauterine embryo distribution

Mammals reproduction is the outcome of a series of complex events, which are driven by synchronized molecular and biochemical procedures in concert with transport phenomena and physical forces. First, the spermatozoa and the ovum are transported to be close to each other in order to allow their fusion which ignites the reproductive chain of events. These transport processes are controlled by the ovary bearing the dominant follicle. The fertilized ovum is then transported to the uterus while cell division takes place within its volume. Within the uterus, the embryo is transported for another few days until the endometrium turns receptive and ready for embryo implantation. Once the embryo's trophoblasts manage to invade into the uterine lining, embryonic development of the new fetus begins, concomitant with the development of the placenta. These biomechanical aspects lead to development of the new field of reproductive bioengineering (Elad and Wildt, 2009; Elad and Young, 2007).

A large volume of knowledge has been accumulated in the past two decades about uterine contractility in nonpregnant women and their role in embryo transport to an optimal site of implantation (Bulletti and De, 2006; Eytan et al., 1999; Fanchin and Ayoubi, 2009; Meirzon et al., 2011). The biomechanical nature of the driving forces that lead to preimplantation intrauterine embryo distribution encouraged development of computerized analysis of *in vivo* ultrasound imaging (Eytan et al., 2001d, 1999) and novel computational models of uterine peristalsis and intrauterine fluid flow (Eytan et al., 2001b; Eytan and Elad, 1999; Yaniv et al., 2003, 2009) in order to decipher this complex process. In addition, *in vitro* laboratory modeling of ET revealed the dependence of the outcome on geometry and posture of the uterus, as well as the clinical protocol of performing the ET procedure (Eytan et al., 2007a,b,c, 2004).

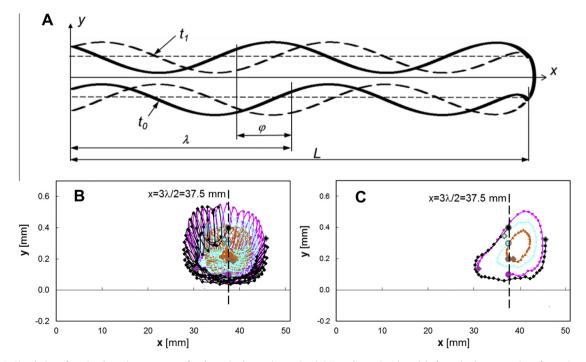


Fig. 5. Simulation of pre-implantation transport of embryos in the uterine cavity. (A) Two-dimensional model of a sagittal cross-section of a uterine cavity which is closed at the fundal end and open towards the cervix; λ is the wavelength, T is the time period and φ is the phase difference between the upper and lower walls. The full line shows the cavity at $t_0 = 0$ while the dashed line shows the cavity at $t_1 = T/4$. (B) The predicted trajectories of embryos (initially located $x = 3\lambda/2$) after 30 cycles presented at consecutive time intervals of T/4 (e.g., 4 times per cycle). (C) The predicted trajectories of embryos as in (B) presented after each full peristaltic cycle.

The computational simulations of intrauterine fluid flow patterns in a closed uterine cavity model can generate the trajectories of massless particles (Yaniv et al., 2009), which may closely mimic the transport of embryos with the intrauterine fluid (Fig. 5A–C). The computed results showed that particles are transported around the initial axial location in small loops that correspond to the frequency of uterine peristalsis (Fig. 5A–C). In the case of idealized uterine peristalsis the embryo will recirculate around its initial location until the endometrium becomes receptive and ready for embryo implantation. During this time window the embryo maintains the course of recirculations until it approaches the wall and the biological aspects of implantation are stimulated. This biophysical approach is in accord with the observations that implantation of the embryo occurs preferentially on the side of the dominant ovary from which the ovum emerged (Kunz et al., 2000). This model also supports some clinical observations after ET procedures that implantation of the embryo occurs in the area where it was placed (Baba et al., 2000).

The biomechanics-based computational models are still at their infant stage as to mimic the actual state of the complex intrauterine environment. However, continuous development of algorithms and increasing computing power makes this emerging direction worth future exploration. In practice, intrauterine shape and geometries are highly variable in different patients, especially in patients with abnormal uterine contraction patterns or fluid conditions. Hence, further improvement of this model by including the different patient physiological/pathological parameters would lead to patient-specific simulations for customized ET protocols. Combining high-resolution ultrasound uterine imaging and patient-customized computational simulations could potentially help improve implantation rate and avoid pregnancy complication associated with intrauterine embryo distribution, such as placenta praevia (Bhide and Thilaganathan, 2004) and vanishing twin syndrome (caused by crowded implantation sites) (Pinborg et al., 2006, 2005). This novel direction, although currently seems too early to be clinically practical, is a promising research filed that requires collaborations among clinicians, reproductive scientists, bioengineers, and computational experts.

5. Closing remarks and perspectives

In summary, the volume of knowledge on pre-implantation intrauterine embryo spacing is continuously growing. However, the detailed cellular and molecular mechanisms controlling the precise process of intrauterine embryo location are still lacking. Future high-resolution noninvasive imaging methods to observe the real-time changes of intrauterine embryo transport will undoubtedly improve our understanding of this process. Also, given that mouse and human share many similarities in the essences of intrauterine environment such as regulated muscular contractions, intrauterine fluid regulation and luminal closure, future use of animal models, especially genetically engineered mice, will undoubtedly help understanding the nature of embryo site selection in human implantation. Physiologically relevant *in vitro* models and refined computational simulation models are needed to assist understanding of the essential process of *in vivo* embryo migration. These multidisciplinary investigations could eventually help develop novel therapeutics to choose an optimal implantation location in women with abnormal uterine anatomy or functions (e.g., abnormal uterine contraction, abnormal uterine fluid environment, etc.) that could lead to abnormal intrauterine embryo distribution, and to improve the success of ET practices after IVF.

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