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NEW RESEARCH HORIZON ***Invited Review***

Embryo–uterine cross-talk during implantation: the role of Wnt signaling†

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ABSTRACT: During mammalian pregnancy, it has been demonstrated that the quality of embryo implantation determines the quality of ongoing pregnancy and fetal development. Recent studies have provided increasing evidence that differential Wnt signaling plays diverse roles in multiple peri-implantation events. This review focuses on recent progress on various aspects of Wnt signaling in preimplantation embryo development, blastocyst activation for implantation and uterine decidualization. Future studies with conditional deletion of Wnt family members are hoped to provide deeper insight on the pathophysiological significance of Wnt proteins on early pregnancy events.

Key words: Wnt signaling / preimplantation embryo development / blastocyst activation / implantation / decidualization

Introduction

Successful implantation requires the embryo's development into an implantation-competent blastocyst and the synchronized transformation of uteri into a receptive stage. Although there have been numerous signaling factors and pathways found to be important for this process (Dey *et al*., 2004; Wang *et al*., 2006), the molecular basis of reciprocal interactions between the blastocyst and the uterus during implantation still remains largely unknown. Recent progress exploiting global genomic microarray screening and increasing number of transgenic mouse models has promised new hopes and strategies to unravel the embryo–uterine dialog during implantation. Among a range of identified signaling pathways, Wnt signaling has recently drawn increasing attention and interest in the peri-implantation event.

Wnt proteins constitute a large family of cysteine-rich secreted molecules that regulate cell–cell interactions during embryogenesis and development in nematodes to mammals (Cadigan *et al*., 1998; Huelsken and Birchmeier, 2001; Van and Berns, 2006). To date, at least 19 *Wnt* genes have been identified in mouse and other vertebrates with 7 in invertebrates. When Wnt proteins bind to two receptors, Frizzled (Fzd, currently 10 members) proteins and lipoprotein receptor-related proteins 5 and 6 (LRP5/6), classic canonical Wnt signaling is activated (Bhanot *et al*., 1996; Pinson *et al*., 2000; Tamai *et al*., 2000; Wehrli *et al*., 2000; Mao *et al*., 2001). As a result, β-catenin, which is encoded by *Ctnnb1* gene is stabilized and accumulates in the cytoplasm, which then translocates into the nucleus and interacts with T-cell/lymphoid enhancer-binding (Tcf/Lef) transcription factors to influence transcription of target genes (Gordon and Nusse, 2006; Willert *et al*., 2006). Also, Wnt proteins can signal through β-catenin-independent (non-canonical) pathways solely via Fzd receptors, regulating Ca^{2+}/planar cell polarity and Rho signaling (Veeman *et al*., 2003; Barrow, 2006). Genetic and biochemical evidence has demonstrated that Wnt activity can be regulated by two main classes of antagonists. The first group consists of Wnt ligand-binding proteins, the secreted Fzd-related proteins (sFRPs), which are structurally similar to the extracellular domains of the Fzd family, which exert their functions by preventing extracellular Wnt ligands from interacting with Fzd receptors (Rattner *et al*., 1997; Xu *et al*., 1998). Alternatively, the second class of antagonists, the Dickkopf (DKK) family proteins, do not prevent Wnt from associating with Fzd receptors but directly interact with LRP5/6 co-receptors to form a ternary structure, resulting in a rapid removal of the cell surface LRP receptors via Kremen-mediated endocytosis (Glinka *et al*., 1998; Fedi *et al*., 1999; Krupnik *et al*., 1999; Bafico *et al*., 2001; Mao *et al*., 2001; Semenov *et al*., 2001). The activation of each of these signaling pathways depends on specific binding of Wnt ligands, receptors, extracellular antagonists and intracellular signaling components, thus determining whether the signaling cascade is driven by canonical or non-canonical pathways.

Previous studies have revealed that Wnt proteins play important roles in various developmental and pathophysiological processes, including embryogenesis and organogenesis, tumorigenesis and homeostasis. Since an intricate interplay between the embryo and the uterus during implantation shares similar features of reciprocal cell–cell

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communications as that during organogenesis, signaling pathways driven by Wnt proteins are likely to participate in this process. In fact, emerging evidence supports the concept that Wnts are important players in multiple peri-implantation events. We herein briefly summarize recent progress on the pathophysiological significance of differential Wnt pathways during preimplantation embryo development, blastocyst implantation and post-implantation uterine decidualization.

Wnt signaling in preimplantation embryo development

Preimplantation embryo development consists of successive cleavage of fertilized zygotes leading to the formation of morulae which undergo compaction before differentiation to blastocysts with activation. Recent reports demonstrated that many Wnt ligands, receptors and related regulators are extensively expressed through mouse preimplantation embryo development (Lloyd *et al*., 2003; Mohamed *et al*., 2004; Wang *et al*., 2004a; Kemp *et al*., 2005). There was also evidence that total and dephosphorylated (active) β-catenin are expressed in early embryos spanning fertilized 1-cell embryos to blastocysts, with total β-catenin primarily localized at the membrane and cytoplasm whereas the active β-catenin localized at the nuclei of embryonic cells. However, β-catenin null mutation studies revealed that lack of zygotic β-catenin does not significantly impair the formation of blastocysts (Haegel *et al*., 1995; Huelsken and Birchmeier, 2001). However, these mouse models failed to preclude the contribution of residual maternal β-catenin during preimplantation embryo development (Haegel *et al*., 1995). Investigation employing conditional elimination of β-catenin in oocytes demonstrated that the zygotes, even with depletion of both maternal and zygotic β-catenin form blastocysts in culture, acknowledging that β-catenin does not play a critical role during preimplantation embryo development (De *et al*., 2004). In this respect, our recent study using the strategy of adenoviral vector (ADV)-mediated DKK1 overexpression for conditional inactivation of nuclear β-catenin signaling or employing small molecular inhibitors of nuclear TCF/β-catenin complexes, demonstrated that silencing of Wnt/β-catenin signaling does not adversely affect the development of preimplantation embryos, further confirming that canonical Wnt-β-catenin pathway is unlikely to be required for preimplantation embryo development (Xie *et al*., 2008). In fact, the diverse expression of Wnt family components during preimplantation embryos (Harwood *et al*., 2008) implies that there are alternative β-catenin-independent Wnt signaling pathways during early embryo development. It is conceivable that differential Wnt proteins may function through MAPK and/or Ca^{2+} pathways, and these pathways are known to be essential for normal preimplantation embryo development (Pey *et al*., 1998; Wang *et al*., 2004b; Xie *et al*., 2005). This concept is further supported by recent observations on dishevelled (Dvl) family proteins, important intermediate transducers of divergent Wnt pathways (Capelluto *et al*., 2002; Itoh *et al*., 2005), showing their potential roles in regulating cell–cell adhesion during preimplantation embryo development (Na *et al*., 2007). Nonetheless, although canonical Wnt activities seem to be dispensable for the preimplantation development of zygotes to blastocysts, definitive roles of differential Wnt pathways during early embryo development remain elusive and future studies are warranted.

Wnt signaling during blastocyst activation, a step towards blastocyst competency for implantation

The blastocyst's state of activity is equally important to the achievement of uterine receptivity in defining the window of implantation (Paria *et al*., 1993; Wang and Dey, 2006). Although a wide range of signaling molecules has been identified to be critical in specifying uterine receptivity for implantation (Wang and Dey, 2006), there is limited information regarding the signaling network that governs blastocyst activation (Paria *et al*., 1998; Wang *et al*., 2003; Hamatani *et al*., 2004). Expression studies have provided substantial evidence that some Wnt ligands may play a role during morula-blastocyst transition and blastocyst activation toward implantation (Mohamed *et al*., 2004). In this respect, recent findings using conditional elimination of β-catenin in oocytes indicated that Wnt/β-catenin signaling, although is not required for blastocyst formation, but is essential for normal blastocyst function during implantation (De *et al*., 2004). For example, female mice with conditional deletion of β-catenin in oocytes produce reduced number of pups when crossbred with wild-type males in comparison with those of wild-type to wild-type mating; whereas this reduction in pup numbers is rescued in females with conditional deletion of both β-catenin and E-cadherin in oocytes (De *et al*., 2004). Considering diverse roles of β-catenin in cellular functions, including its association with E-cadherin in adherens junctional complexes and functioning as an intermediate in canonical Wnt pathways, this study suspected that paternal derived β-catenin in blastocysts with maternal β-catenin depletion is primarily incorporated into adherens junctions, causing insufficiency for nuclear Wnt signaling and thereby leading to loss of blastocysts during the periimplantation period. In contrast, simultaneous depletion of β-catenin and E-cadherin restores nuclear β-catenin signaling in blastocysts, because in the presence of less E-cadherin, more β-catenin is available for nuclear Wnt signaling (De *et al*., 2004). Moreover, our recent investigation, using the strategy of DKK1-mediated functional inhibition of nuclear β-catenin signaling and small molecule inhibitors of Wnt signaling, demonstrated silencing canonical Wnt/β-catenin signaling does not adversely affect the uterine preparation for receptivity, but remarkably blocks blastocyst competency to implant. Employing the physiologically relevant delayed implantation model and trophoblast stem cells in culture, we further observed that a coordinated activation of canonical Wnt-β-catenin signaling with attenuation of the non-canonical Wnt-RhoA signaling pathway ensures blastocyst competency to implantation (Xie *et al*., 2008). These findings constitute direct evidence that Wnt signaling is at least one pathway determining blastocyst competency for implantation. However, it remains elusive regarding a definitive molecular hierarchy of Wnts among other signaling molecules in ensuring blastocyst activation for implantation. Early studies in mice have demonstrated that catecholestrogens formed locally in the uterus from the primary estrogen participate in blastocyst activation during the peri-implantation period (Paria *et al*., 1998). Since catecholestrogens can induce canonical Wnt activation in the uterus (Hou *et al*., 2004; Ray *et al*., 2008), it would be interesting to study potential interactions between catecholestrogen

and Wnt signaling in blastocysts during implantation to further reveal underlying molecular mechanisms governing blastocyst activation.

Embryo-induced uterine Wnt/β-catenin signaling toward implantation

It is generally accepted that the embryo plays an active role in regulating uterine preparation toward implantation via its secreted paracrine and juxtacrine factors (Paria *et al*., 2001). For example, implanting blastocysts exhibit up-regulated expression of heparin-binding epidermal growth factor-like growth factor (HB-EGF), which via an autoinduction loop induces its own gene expression in the uterine epithelium solely at the site of blastocyst apposition (Hamatani *et al*., 2004). With respect to Wnt-mediated embryo–uterine cross-talk during implantation, a recent study using a TCF/Lef-LacZ reporter mouse, which faithfully monitors activation of Wnt/β-catenin pathway, demonstrated that the uterine Wnt/β-catenin signaling is transiently and strictly induced at the prospective site of embryo attachment immediately before implantation (Mohamed *et al*., 2005). This timely Wnt activation requires the presence of active blastocysts as well as the preimplantation estrogen secretion (Mohamed *et al*., 2005). Furthermore, an intraluminal delivery of Wnt7a protein, a Wnt ligand expressed by the active blastocysts (Mohamed *et al*., 2004, 2005) can induce uterine Wnt/β-catenin activation, equivalent to that by the living blastocyst; whereas intrauterine co-administration of canonical Wnt signaling inhibitor sFRP-2 inhibits uterine Wnt/β-catenin activation and impairs implantation. These results support the notion that Wnt/β-catenin signaling pathway plays a critical role in coordinating embryo–uterus preparation toward successful implantation. In addition, it has been reported that Wnt7a is transiently expressed in the ovine uterine luminal epithelium during the peri-implantation period, and can induce differential Wnt activities in trophoblast cells (Hayashi *et al*., 2007), suggesting Wnt signaling also plays an important role during embryo–uterine interactions during early pregnancy in sheep (Kim *et al*., 2003; Hayashi *et al*., 2007). However, it remains to be explored whether uterine activation of Wnt pathways is seeded by embryonic Wnt ligands or other signaling molecules. Recent *in vitro* fertilization–embryo transfer studies in women have observed that the implantation of every embryo facilitates the chances of the remaining embryos implanting in the uterus, implying that implanting embryos are actively involved in the process of implantation and fine tuning the endometrium to become more receptive for the implanting embryo (Matorras *et al*., 2005; Pietras *et al*., 2005). Thus, it will be interesting to investigate further the significance of Wnt signaling in embryo–uterine cross-talk in model systems closer to humans.

Wnt signaling in regulating embryo spacing?

In polyovulatory species, embryos tend to be equally spaced with each other along the uterine horn before the initiation of implantation. This well-organized embryo apposition process helps prevent embryo overcrowding and preclude the possibility of consequent loss of embryos (Wimsatt, 1975). Although the phenomenon of embryo spacing has been noticed for more than 100 years, there is still very limited information available regarding its underlying cellular and molecular basis. It was generally thought that proper timely regulated uterine muscular contraction account for normal embryo spacing, which has been suggested to linked with prostaglandin signaling (Wellstead *et al*., 1989). Recent studies using null mutation mouse models further revealed potential roles of lysophosphatidic acid (LPA) receptor (LPA3), cytosolic phospholipase A2α (cPLA2α) and respective lipid mediators in embryo spacing (Song *et al*., 2002; Ye *et al*., 2005; Hama *et al*., 2007). However, it is not known yet how this uterine epithelium-derived LPA3/cPLA2α signaling would influence the function of heterogeneous uterine cells, including the muscular cells for proper embryo apposition. Previous observations of failure to restore normal embryo spacing in mice missing LPA3 or cPLA2α by prostaglandins implicated that there are alternative signaling molecules involved in the regulation of proper embryo spacing. In this regard, recent studies demonstrated that Wnt/β-catenin signaling is transiently activated in circular smooth muscle of early Day 4 pregnant uterus, forming evenly spaced bands along the uterine horn from the oviduct end toward the cervix end (Mohamed *et al*., 2005), raising the possibility that Wnt/β-catenin signaling may play a role ensuring normal embryo spacing in the uterus. This unique pattern of uterine Wnt/β-catenin activation requires the advent of embryos and disappears in the muscular layer prior to the onset of blastocyst attachment. A possible explanation would be that the blastocyst emits a signal(s) that directly or indirectly activates the Wnt/β-catenin signaling pathway in circular smooth muscular cells, thus to regulate normal uterine contraction. Indeed, there have been reports demonstrating the cross-talk between Wnt and prostaglandin signaling in other systems (Wang *et al*., 2004c), suggesting that these signaling pathways might coordinately regulate the process of embryo spacing before the initiation of attachment reaction. However, although there is evidence showing Wnt/β-catenin pathway actions on muscular cells, none of them mention its roles in modulating muscular contractility. The notion of Wnt proteins regulating embryo spacing still awaits further proof in a more physiological environment.

Wnt signaling in uterine decidualization

Recent studies have found that many Wnt ligands and Wnt signaling-related genes are dynamically expressed in the uterine stroma during the process of uterine decidualization (Paria *et al*., 2001; Daikoku *et al*., 2004; Hayashi *et al*., 2007; Peng *et al*., 2008; Zhang *et al*., 2008). For example, in pregnant mouse uterus, Wnt4 expression is first undetectable in the uterus on the morning of Day 4, but increased in the stroma surrounding the embryo with the onset of attachment reaction at midnight of Day 4, and further enhanced on Day 5 and beyond. By Day 7, Wnt4 is strongly expressed throughout the whole deciduas (Paria *et al*., 2001). This spatiotemporal expression of Wnt4 implicates that Wnt4-driven signaling plays an important role during implantation and decidualization. Interestingly, this highlighted expression of Wnt4 coincides with the expression of bone morphogenetic protein 2 (BMP2) (Paria *et al*.,

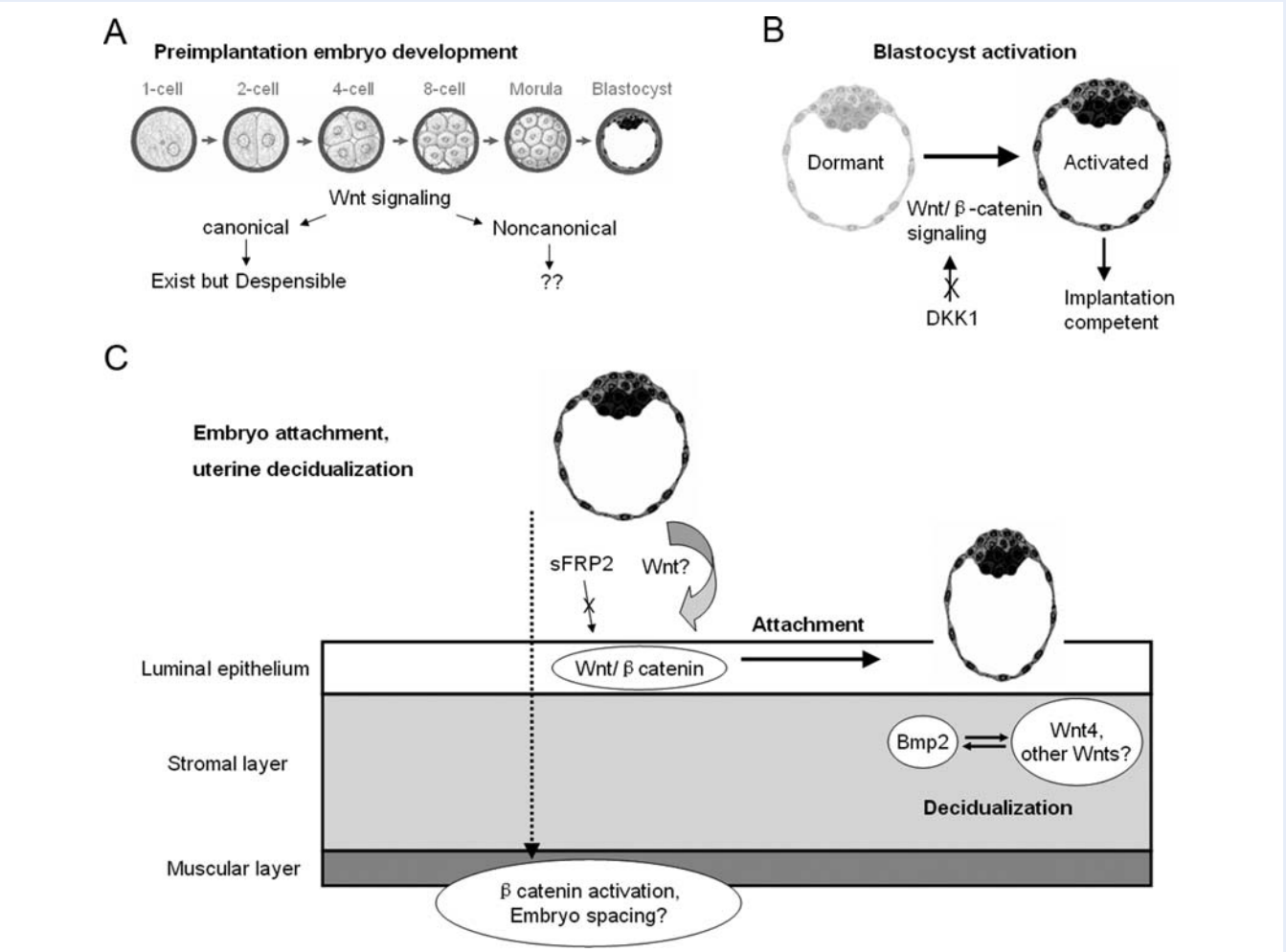

Figure 1 Wnt signaling in peri-implantation events.
(**A**) Peri-implantation embryo development from zygote to blastocyst involves extensive expression of many Wnt ligands, receptors and related regulators. However, an exact role of Wnt signaling during these processes still waits future identification. (**B**) Blastocyst activation is a crucial step synchronizing the transformation of uterine receptivity for normal embryo implantation. Wnt signaling has been demonstrated as a necessary pathway that ensures this process determining blastocyst competence for implantation. (**C**) Wnt/β-catenin signaling is required for normal embryo–uterine interaction to initiate embryo implantation. Wnt signaling is also actively involved in the process of post-implantation decidualization. The possible relationship between Wnt signaling and embryo spacing is intriguing and warrants future investigation. DKK, Dickkopf family proteins (Wnt antagonists); sFRP, secreted Fzd-related protein (Wnt ligand-binding protein).

2001), which has been shown to be a critical factor for normal uterine decidual response in mice (Lee *et al*., 2007). In fact, recent evidence suggests that Wnt4 may function in downstream signaling of BMP2 during progesterone-induced stromal decidualization in both cultured mice and human endometrial stromal cells (Li *et al*., 2007). Most recent observations that mice with conditional deletion of β-catenin or constitutive overexpression of dominant stabilizing β-catenin in mouse uteri exhibit decidualization defects (Jeong *et al*., 2009) further support the concept that normal Wnt activities are critical for decidualization success. Besides the findings in mice, there is also evidence that progesterone can regulate Wnt signaling in the rat and ovine uterine stroma by selectively up- or down-regulating specific Wnt signaling components (Rider *et al*., 2006; Satterfield *et al*., 2008), In addition, several Wnt family members and their inhibitors are uniquely expressed in human endometrium during the menstrual cycle (Tulac *et al*., 2003, 2006). However, there are also reports showing that trophoblasts can emit paracrine factors down-regulating endometrial Wnt activities during decidualization (Hess *et al*., 2007). Nonetheless, a precisely regulated Wnt signaling is essential for normal uterine decidualization.

Conclusion

Despite recent progress in elucidating Wnt proteins' roles in peri-implantation events (Fig. 1), the hierarchal relationship between Wnt signaling and other implantation-related molecules, especially in the context of *in vivo* animal models is intriguing and warrants further investigation. Only limited number of Wnt family knockout mice studies have revealed reproductive-related phenotypes so far (Table I), which is probably due to the fact that genome-wide deletion

Table I Reproduction-related phenotype in Wnt mutant mice

Gene name	Reproduction-related phenotypes	References
Wnt2	Placental defects	Monkley *et al.* (1996)
Wnt4	Defective female reproductive tract development (absence Mullerian Duct); ectopic testosterone synthesis in females	Vainio *et al.* (1999)
	Overexpression disrupts normal testicular vasculature and inhibits testosterone synthesis in male	Jordan *et al.* (2003)
	Impaired sex determination	Kim *et al.* (2006)
Wnt5a	Defects in posterior growth of the female reproductive tract	Mericskay *et al.* (2004)
Wnt7a	Female infertility; failure regression of the Mullerian duct because the receptor for Mullerian-inhibiting substance is not expressed	Parr *et al.* (1998)
Wnt7b	Placental developmental defects	Parr *et al.* (2001)
Fzd5	Abnormal yolk sac development and placental angiogenesis	Ishikawa *et al.* (2001)
Tcf1 (official name Tcf7)	Defects in the formation of the placenta and in the development of limb buds in double mutant with Lef-1	Galceran *et al.* (1999)
β-catenin	Maternal deletion of β-catenin in oocytes does not impair blastocysts formation, but shows compromised pregnancy outcome	De *et al.* (2004)
	Dominant β-catenin expression in granulosa cells resulted in ovarian granulosa cell tumor	Boerboom *et al.* (2005)
	Conditional deletion of beta-catenin in embryonic Müllerian duct result in uterine developmental abnormalities	Arango *et al.* (2005)
	Stabilization of Wnt/β-catenin signaling in primordial germ cells (PGCs) resulted in germ cell deficiency	Kimura *et al.* (2006)
	Uterine conditional deletion or overexpression of β-catenin result in fertility defect with impaired decidualization, also with abnormal endometrial epithelial differentiation	Jeong *et al.* (2009)

of many Wnt family-related genes result in embryonic lethality (Aoki *et al.*, 2008 and see Wnt home page: http://www.stanford.edu/~rnusse/wntwindow.html), hampering studies on Wnt functions during embryo implantation and uterine decidualization. The wide use of Cre-Loxp transgenic mouse models will be a feasible strategy to further explore the roles of *Wnt* genes during implantation. For example, many previous inaccessible developmental genes such as India hedgehog *(IHH)* and *BMP2* have recently been proved to be critical for normal implantation (Lee *et al.*, 2006, 2007). Future studies using conditional deletion of *Wnt* family members will provide valuable information toward more comprehensive understanding of Wnt signaling in peri-implantation events.

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