Focus on Implantation

Animal models of implantation

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Abstract

Implantation is an intricately timed event necessary in the process of viviparous birth that allows mammals to nourish and protect their young during early development. Human implantation begins when the blastocyst both assumes a fixed position in the uterus and establishes a more intimate relationship with the endometrium. Due to the impracticalities of studying implantation in humans, animal models are necessary to decipher the molecular and mechanical events of this process. This review will discuss the differences in implantation between different animal models and describe how these differences can be utilized to investigate discrete implantation stages. In addition, factors that have been shown to be involved in implantation in the human and other various animal models including growth factors, cytokines, modulators of cell adhesion, and developmental factors will be discussed, and examples from each will be given.

Introduction

Human implantation begins when the blastocyst both assumes a fixed position in the uterus and establishes a more intimate relationship with the endometrium. In order for this relationship to be established, an ordered succession of events must occur. The events of implantation include: apposition of the blastocyst to the uterine luminal epithelium, adhesion to the epithelium, penetration through the epithelium and basal lamina and invasion into the stromal vasculature (Enders et al. 1986). It is not possible, for both practical and ethical reasons, to physiologically study implantation in large numbers of humans. Therefore, animal models are necessary to decipher both the molecular and mechanical events associated with implantation. Although the eventual objective of implantation, to bring the conceptus into contact with the maternal blood supply, is the same in all mammals, the different animal models in which implantation has been studied have shown wide mechanistic variation. The study of these differences and similarities allows us to gain insight into cellular and molecular interactions that occur as part of implantation. In the sections below, shared mechanisms of implantation will be defined and the mechanistic differences in implantation between different animal models will be illustrated. It will be shown how these differences can be utilized in the investigation of discrete implantation stages. Furthermore, information on different factors that may be involved in implantation across species, including humans, will be given, with examples from each class of factors.

Mechanisms of implantation in animal models

Implantation is an intricately timed event necessary in the process of viviparous birth that allows mammals to nourish and protect their young during early development. Although there are numerous differences in the mechanism of implantation between species, the processes between fertilization and the initiation of implantation seem to follow a well-conserved autonomous process (McLaren 1990). After fertilization, a single cell embryo doubles to two cells, four cells, eight cells, and then becomes a morula. Several more rounds of mitotic division leads to the formation of the blastocyst, which is comprised of differentiated tissues. In most mammals, the blastocyst is comprised of a layer of trophectoderm cells that will give rise to the placenta and the inner cell mass (ICM) that will give rise to the embryo (McLaren 1990). After shedding the zona pellucida, the blastocyst becomes implantation competent. This preimplantation stage varies in duration between species. In mice, implantation occurs 4 days post coitum, humans average 9 days, and in cows implantation does not occur until 30 days after fertilization (McLaren 1990, Wilcox et al. 1999).

Implantation is the process in which the blastocyst physically and physiologically comes into intimate contact...
with the uterus. Based on the different types of blastocyst–uterine cell interactions, implantation has been classified into three broad categories: centric, eccentric and interstitial (Wimsatt 1975). Centric implantation occurs when the blastocyst is able to grow large and form ample surface contact to fuse with the luminal epithelium without penetrating through it. Rabbits, dogs, domestic animals (cows, pigs, and sheep) and many marsupials have centric implantation. In eccentric implantation, the luminal epithelium forms an invagination to surround the trophoblast. Mice, rats and hamsters have eccentric implantation. The third type of implantation is interstitial, in which the trophoblast passes through the luminal epithelium to invade the endometrial stroma and become imbedded into the wall of the uterus. Implantation in humans and guinea pigs has been classified into this category (Wimsatt 1975). Regardless of the type, the purpose of implantation is to bring the maternal blood supply into contact with the developing embryonic blood vessels. At the site of attachment, there is a concomitant increase in the permeability of the blood vessels that can be visualized in mice and rats by injection of a large molecular weight blue dye (Dey 2003). At this site, the endometrial stromal cells undergo the decidual reaction, in which they proliferate and differentiate to form morphologically distinct decidual cells. The decidual cells together comprise the decidua. A description of the mechanisms of implantation in several animal models follows.

**Mice and rats**

Mice and rats demonstrate extremely rapid eccentric implantation with apposition, attachment and invagination of the uterine epithelium occurring in 6 hours. After the loss of the zona pellucida, the uterine lumen closes down on the blastocyst and brings it into close apposition (reviewed in Dey et al. 2004). The blastocyst attaches to the anti-mesometrial side of the endometrium with its ICM pointed in the mesometrial direction. The epithelial layer in contact with the blastocyst then is phagocytized by the polytene cells from the walls of the blastocyst, starting epithelial penetration. Trophoblastic cells penetrate through the residual uterine luminal basal lamina. The forming decidual area invaginates to create a pocket for the blastocyst and orients it so that its ICM points in the mesometrial direction. Differentiation and degradation of the primary decidual area brings the trophoblast into contact with the maternal blood supply forming the placenta and providing room for the conceptus to grow (Carson et al. 2000). Certain rodents (mice and rats) can undergo delayed implantation in which the uterus remains in a quiescent state causing embryos in a blastocyst state to remain dormant. This state can be artificially stimulated by ovariectomy before the morning of day 4 (day 1 is determined by presence of the vaginal plug after mating) and treatment with progesterone (P4). A single injection of estrogen (E2) into the P4-primed uterus will induce implantation. In addition, the decidual reaction can also be artificially stimulated in mice and rats by trauma (intraluminal infusion of oil or scratching with a needle) to the uterus, suggesting that the decidual response is an intrinsic ability of uterine stromal cells (reviewed in Dey et al. 2004). Due to the rapidity of apposition, attachment and invagination of the luminal epithelium, mice and rats are not good candidate models for understanding the physical mechanisms of early implantation. However, since the decidual response can be elicited in a reproducible manner in the absence of trophoblastic attachment, and decidualized regions in pregnant mice are easily discernable, mice and rats make a good model to study the mechanism of decidualization.

The real power of the mouse model is the ability to exploit the vast knowledge of mouse genetics by genetically overexpressing or ablating genes. Although there are substantial mechanistic differences between mouse and human implantation, gene ablation in the mouse has proven to be a powerful tool in elucidating gene function during implantation.

**Guinea pigs**

As mentioned earlier, the guinea pig, like the human, undergoes interstitial rather than eccentric implantation. While the blastocyst is still in the lumen, it forms an implantation cone at the opposite side of the blastocyst from the ICM. This implantation cone is composed of syncytiotrophoblast cells and sends processes through the zona pellucida to adhere to the uterine luminal epithelium (Enders & Schlafke 1969). The processes penetrate between the basal lamina as well as uterine epithelial cells, therefore, the blastocyst is able to intrude directly into the endometrial stroma, sloughing the zona pellucida into the lumen as it progresses through the epithelium. After the blastocyst is ensconced into the endometrial stroma, the decidualization of the stroma begins to occur in a similar manner to mice and rats. The direct entry of the blastocyst into the endometrial stroma makes the guinea pig a useful model for elucidating the mechanism of transepithelial penetration, despite difficulties in locating individual implantation sites (Enders 2000).

**Rabbits**

The rabbit is an example of the centric, or fusion, type of implantation in which the blastocyst adheres solely to the apices of the epithelial cells. The rabbit trophectoderm creates large knob-like projections that adhere and fuse to the apical surface of epithelial cells (Enders & Schlafke 1971). Due to the precisely timed ovulation (10 hours after mating) and well-documented time of apposition and attachment (day 6.5), as well as, the ability of rabbit trophoblasts to attach to in vitro cultured epithelium, the rabbit is a good model to study apical cell adhesion (Hoffman et al. 1998).
**Pigs**

Implantation in the pig is characterized by a lengthy pre-attachment period that includes migration and spacing of embryos within the uterus. In addition, remodeling of the embryo takes place in which the spherical blastocyst elongates to form a filamentous conceptus of about 10 cm in length with a centrally positioned embryonic disc (Bazer 1975, Geisert & Yelich 1997, Burghardt et al. 2002). The pig demonstrates epitheliocorial placentation development, as implantation remains superficial, and all three maternal and three embryonic cellular layers separating the maternal and fetal circulations remain intact (McLaren 1990, Wooding 1992). Despite the lack of perturbation of maternal tissue, the trophoblast and luminal epithelium become closely interlocked. The blood vessels that develop from the blastocyst and those from the uterus only indent their respective epithelia, so the integrity of both the maternal and fetal tissues is not disrupted (reviewed in Carson et al. 2000). The prolonged apposition and attachment phase, without invasion into the luminal epithelium confounding interpretation of results, makes the pig a good candidate model to study these early phases of implantation.

**Sheep and cows**

In adult sheep and cows, the uteri are comprised of large caruncules, stromal glandular protrusions covered by luminal epithelium, and intercaruncular areas containing considerable amounts of glandular epithelium. The caruncular areas are the sites of implantation (reviewed in Gray et al. 2001). Sheep, like multiparous pigs, undergo a prolonged preimplantation stage characterized by migration and elongation into a filamentous conceptus. In sheep and cows, there is a formation of individual intraepithelial binucleate cells that form 15–20% of the trophectoderm and appose the caruncular sites of initial attachment to the uterine epithelium (Wooding 1984). These cells fuse with luminal epithelial cells and form placentomes, which serve to mediate gas and micronutrient exchange through the placenta (Wooding 1992). Like the pig, implantation in sheep and cows is epitheliocorial, and these models serve as futher candidates for the study of apposition and attachment.

**Primates**

Although implantation in other animal models, such as mice and rats has distinct advantages in monetary and temporal cost, vast amounts of genetic information, and the ability to be genetically modified, they remain inherently limited in their ability to elucidate the physiological mechanisms of human implantation. However, studies in non-human primates have shown high fidelity to human implantation, suggesting their potential as models of early pregnancy. In primates with a uterus similar to humans (a simplex uterus with slot-like lumen), the lumen is closed at the time of adhesion of the blastocyst to the luminal epithelium (Enders 2000). Ultrasonographic techniques have been developed to study early gestational events (Tarantal & Hendrickx 1988) and it has been shown that in the implantation of the cynomolgus macaque, the ventral and dorsal walls of the uterus are in close contact surrounding the blastocyst (Tarantal et al. 1997). Extrapolating back from the orientation of the trophoblast as it invades the epithelium, Adams and his colleagues (Adams et al. 1956) hypothesized that the human trophoblast adheres to the endometrium adjacent with the ICM. This finding is in agreement with other primate species in which early attachment has been studied. In the baboon, blastocyst flushed during the time of implantation show syncytial trophoblast and irregular projections, presumably necessary for adhesion to endometrial epithelial cells adjacent to the ICM (Enders et al. 1989). Likewise, in the cynomolgus macaque, the marmoset monkey and the baboon, the syncyial trophoblast forms adjacent to the ICM, and it is this differentiated trophoblast lineage that penetrates the epithelium by invading between uterine epithelial cells (Enders et al. 1983, Smith et al. 1987, Jones et al. 2001). With the exception of the marmoset and rhesus monkey, these early implantation studies in non-human primates are relatively difficult to perform given the low fecundity of the species involved and difficulty in ascertaining early pregnancy. In the marmoset and rhesus monkey, the process of epithelial penetration takes several days and can be ascertained by circulating P4 levels. The blastocyst remains superficial as the implantation site spreads peripherally with the syncyial trophoblast repeatedly penetrating the luminal epithelial cells (Enders et al. 1983, Enders & Lopata 1999). Due to the formation of the large spreading implantation site composed of cytotrophoblasts and syncyial trophoblasts, this stage is also known as the trophoblastic plate stage. The capability to determine pregnancy early and the repeated observable penetration of the uterine lumen make the marmoset and rhesus monkey good primate models to study early implantation events.

After the trophoblastic plate stage, the lacunar or pre-villous stage follows and is characterized by the formation of connected syncyial trophoblast-lined lacunae that eventually become filled with maternal blood (Enders 2000). Studies in the macaque show that the syncyial trophoblast penetrates the endothelium of the superficial capillary bed in order to link the blood supply with the lacunae (Enders et al. 1989). Therefore, due to ultrasonographic techniques that can determine implantation sites, large preimplantation blastocyst (Enders & Blankenship 1999), and the easily visible invasion of syncyial trophoblast into the endometrial blood vessels, the macaque is a strong candidate animal model for studying late implantation events.
Implantation factors found in animal models with clinical significance in humans

Ovarian hormones and cognate receptors

In all the animal models of implantation the uterus is able to undergo a transformation into an altered state, in which the blastocysts are capable of attaching and receiving signals with the uterus in order to facilitate apposition, attachment, and intimate physical and physiological contact with the uterus. The primary factors that stipulate endometrial receptivity are the ovarian steroids, E2 and/or P4. These steroids are known to exert their effect primarily through their cognate nuclear receptors, E2 receptor (ER) and P4 receptor (PR). The physiological effect of ovarian steroids on the uterus are best understood in the mouse, where several gene targeting experiments have elucidated the roles of these receptors in uterine function.

Receptivity of the mouse endometrium is steroid dependent, largely due to the combined actions of both E2 and P4. In adult mice, E2 causes epithelial cell proliferation, while the combined action of E2 and P4 is necessary to drive proliferation in stromal cells. During normal pregnancy in mice, a pre-ovulatory E2 surge stimulates uterine epithelial cell proliferation on day 1. On day 2, with the withdrawal of E2 ovarian steroid hormone, a large number of epithelial cells undergo apoptosis. With the newly formed corpora lutea on day 3, rising P4 levels initiate uterine stromal cell proliferation. With P4 priming of the uterus, preimplantation E2 secretion on day 4 further stimulates uterine stromal proliferation and differentiation, rendering the uterus to the receptive state for implantation. (Huet-Hudson et al. 1989). In the mouse, E2 and P4 act primarily through their cognate nuclear receptors of E2 receptor alpha (ER-α) and beta (ER-β), and P4 receptor A (PR-A) and B (PR-B) respectively, to alter gene expression levels. After ovariectomy followed by E2 treatment, ER-β knockout mice have uterine gene expression profiles similar to wild type. Therefore, E2 modulation of gene expression in the uterus is primarily through ER-α and not ER-β (Hewitt et al. 2003). ER-α null mice are infertile due to pleiotropic abnormalities of the reproductive tract including hypoplastic uteri and hyperemic ovaries (Curtis et al. 1999). The inability of transferred blastocysts to implant into uteri of ER-α null mice that have been exogenously treated with E2 and P4, demonstrate that the ER-α is necessary for support of implantation (Curtis Hewitt et al. 2002). Although ER-α is known to modulate PR levels, ER-α null mice are able to show normal physiological responses upon P4 treatment, further defining the discrete functions of E2 and P4 on the uterus. Although decidualization is E2 dependent in wildtype mice, severe trauma, provided by an intraluminal oil infusion, causes the ER-α null mice to undergo a decidual response, including up-regulation of related genes (Curtis Hewitt et al. 2002). Since ER-α is not necessary for the decidual reaction, but ER-α null animals fail to implant, it has been hypothesized that E2 action in the mouse is necessary for attachment. P4 receptor null animals also have severe reproductive tract abnormalities including uterine hyperplasia and inflammation (Lydon et al. 1995). Selective ablation of the PR isomers reveals PR-A and PR-B are functionally distinct mediators of P4 action. Uteri from female PR-A null mice fail to undergo a decidual reaction, whereas PR-B null mice have normal uterine function (Mulac-Jericevic et al. 2000, 2003). Therefore, the targeted disruptions of ER and PR have established their necessity in uterine function in the mouse.

Although both ovarian E2 and P4 are necessary for implantation into the mouse and rat (Canivenc 1956 and Chambon 1949 as reviewed in Carson et al. 2000), an absolute requirement for E2 in some species is questionable. Although early studies in ovariectomized guinea pigs suggest that only P4 is necessary for implantation (Deanesly 1960), more recent experimental evidence suggests that E2 may also play a role in guinea pig implantation (Thapar et al. 1988, Makker et al. 1994). A similar debate has arisen with implantation, over the necessity for E2, in the rhesus monkey (reviewed in Carson et al. 2000), whereas E2 appears to be crucial in the macaque (Moudgal & Ravindranath 1989).

The pleiotropic effects of ovarian steroids have also been used to create additional animal models to study implantation. In the first month after birth, the sheep endometrium develops from a simple tubular lumen to its adult morphology that includes intercaruncular regions filled with uterine glands (Wiley et al. 1987). Since the developmental cue for gland formation is the withdrawal of P4 from the prenatal environment, by ovariectomizing newborn ewes and implanting a 19-norprogestin implant the developmental cue of P4 withdrawal was removed and uterine glands failed to develop (Bartol et al. 1988). This approach of prolonged P4 exposure into neonatal ewes has lead to the development of the ovine uterine gland knockout (UGKO) animal (Spencer et al. 1999). This model has been useful in rapidly identifying genes expressed by the endometrial epithelium (Gray et al. 2002).

In all of the animal models studied, global changes in gene expression induced by the ovarian hormones, especially P4, are critical for implantation and the maintenance of pregnancy. However, the downstream targets of P4 remain relatively unexplored. In the past decade, the use of gene expression screening techniques, including microarray analysis, has begun to identify factors whose expression is stimulated by P4. A few of these target genes that have been shown to have functional significance in implantation include calcitonin (Ding et al. 1994), Indian hedgehog (IHH) (Takamoto et al. 2001), and amphiregulin (Ar)(Das et al. 1995). Intraluminal injection of antisense oligonucleotides to calcitonin into the rat uterus leads to drastically reduced numbers of implantation sites (Zhu et al. 1998). Treatment with recombinant hedgehog protein causes proliferation of mesenchyme
cells (Matsumoto et al. 2002b), and AR antisense oligonucleotides delay blastocyst formation in vitro (Tsark et al. 1997). However, much work remains to be done in the identification and characterization of ovarian steroid regulated genes in the uterus.

**Growth factors**

**Epidermal growth factor (EGF) family members**

EGF family members are transmembrane proteins that undergo proteolytic cleavage to release their mature form into extracellular space. The common structural domain shared by these family members is a 40–60 amino acid domain characterized by six cysteine residues forming three disulphide bonds (Savage et al. 1973). The EGF members signal through a family of receptor tyrosine kinases known as the ErbB family that is comprised of four distinct receptors: EGFR/ErbB-1, HER2/ErbB-2, HER3/ErbB-3 and HER4/ErbB-4. These transmembrane receptors are composed of an extracellular ligand binding domain and a cytoplasmic domain (Ullrich & Schlessinger 1990). This structure allows signals to transverse the cellular membrane and lead to a signal transduction cascade that regulates diverse functions, including cell proliferation, survival, adhesion, migration and differentiation (Yarden 2001). In the mouse uterus, many EGF family members are expressed at the time of implantation. Transforming growth factor-α (TGF-α) is expressed in the luminal epithelium throughout the uterus during preimplantation (Paria et al. 1994). β-cellulin (BTC), epiregulin (Er) and neudifferentiating factors (NDFs) share an expression pattern and are first expressed in the luminal epithelium and underlying stroma at the site of blastocyst attachment (Das et al. 1997b, Reese et al. 1998). AR is a P4 regulated gene that is highly upregulated in uterine epithelium prior to blastocyst attachment on day 4 (Das et al. 1995). The expression of heparin binding EGF (HB-EGF) appears to be significantly related to implantation. HB-EGF is expressed in the luminal epithelium solely at the presumptive sites of blastocyst attachment (Das et al. 1994). Mouse blastocysts show expression of ErbB-1 (Paria et al. 1993), ErbB-2 (Dey et al. 2004) and ErbB-4 (Paria et al. 1999). The expression of the EGF family ligands in the receptive uterus and the receptors in the blastocyst suggest a possible paracrine interaction that may be necessary for attachment.

Other animal models demonstrate that EGF uterine expression is largely conserved between species. In the guinea pig, although the expression pattern of EGF family members has not been studied, EGF treatment, but not E2 or P4 treatment, of guinea pig endometrial stroma cells in culture produces proliferation (Ordener et al. 1993). This result suggests functional EGF pathways in the guinea pig. In the pig, AR is highly upregulated in the preimplantation period in much the same manner as the mouse (Kim, et al. 2003). However, HB-EGF is not as restricted in expression, and can be found in the cycling, as well as, pregnant uterus (Kennedy et al. 1994). The sheep endometrium produces high levels of TGF-α at the time of implantation with EGF-R localized on the trophoderm (Tamada et al. 2002). In the rhesus monkey, several EGF family members including EGF, TGF-α, and HB-EGF as well as EGF-R were detected in the endometrium, with peak expression levels at the time of implantation (Yoo et al. 2000).

In spite of the conserved expression pattern between species that appears to be correlated with implantation, there is relatively little known about the function of these proteins in implantation. Gene targeting experiments have demonstrated that mice null for EGF, AR (Luetke et al. 1999), or TGF-α (Mann et al. 1993) are fertile. In addition, compound knockout mice for all three of these alleles are still fertile (Troyer et al. 2001). Although the majority of HB-EGF null mice die in early postnatal life due to heart malformations (Iwamoto et al. 2003), there is substantial evidence for a role of HB-EGF in implantation. Beads saturated with HB-EGF and transplanted into the lumen of pseudopregnant females are sufficient to initiate decidualization that closely reflects true blastocyst implantation (Paria et al. 2001).

Currently, little is known about the relationship between EGF family members and human implantation. Showing conservation with the mouse, HB-EGF peaks at the window of implantation (Yoo et al. 1997) and ErbB4 is expressed in the trophoderm in perimplantation human blastocysts (Chobotova et al. 2002). In addition, correlative links between elevated expression of Ar and TGF-α with endometrial cancers have been made (Pfeiffer et al. 1997).

**Vascular endothelial growth factor (VEGF)**

An important stage in embryo implantation in all mammals includes the connection of the fetal and maternal blood supplies. For this connection to occur, there must be dramatic growth and remodeling of the endometrial vasculature. VEGF-A is a homodimeric glycoprotein first discovered as an endothelial cell mitogen and is also known as vascular permeability factor due to its ability to induce vascular leakage in guinea pig skin (Ferrara & Davis-Smyth 1997), may be important in this process. Five human VEGF-A isoforms: VEGF121, VEGF145, VEGF165, VEGF189, and VEGF206 (denoted by number of amino acids) are generated through alternative splicing of a single gene (Tischer et al. 1991). The mouse VEGF-A gene undergoes similar splicing leading to the generation of VEGF120, VEGF164, and VEGF188. The murine VEGFs are shorter than the respective human VEGFs by one amino acid. The different isoforms of VEGF have distinctive properties due to this molecular heterogeneity. Native VEGF (VEGF165) is a secreted protein that remains largely bound to the cell surface and extracellular matrix that is able to bind heparin. VEGF121 does not bind heparin and is freely soluble, while VEGF189 and VEGF206 remain almost completely bound to the cellular...
membrane. VEGF165 and VEGF121 are the dominant forms in humans, although it has been shown that the human endometrium synthesizes all the splice variants of VEGF-A (Charnock-Jones et al. 1993). VEGF-A effects are predominantly mediated through two tyrosine kinase receptors: VEGFR-1 (fms-like tyrosine kinase [Flt-1]) and VEGFR-2 (also known as fetal liver kinase-1 [Flk-1] in the human or kinase domain region [KDR] in the mouse). VEGFR-2 is the major positive regulator of VEGF signaling to affect an increase in angiogenesis, vasculization and vasodilation. Ablation of specific components of the VEGF signal transduction cascade shows their necessity for vascular development as demonstrated in VEGF-A heterozygous, VEGFR-1 null, and VEGFR-2 null mice which are all lethal, early in embryonic life (Fong et al. 1995, Shalaby et al. 1995, Ferrara et al. 1996).

The uterine expression of VEGF has been well characterized in a number of animal models. The human VEGF promoter has been shown to be regulated by ER through a variant response element (Mueller et al. 2000), and increased in vivo expression in the sheep and baboon show this regulation is conserved across species (Reynolds et al. 1998, Albrecht et al. 2003). In the mouse, VEGF RNA expression is induced in the luminal epithelium on days 1 and 2, presumably due to high ovarian E2 levels. On day 3, a low level of VEGF can be visualized in the stroma. In days 4–5, VEGF can be seen in the luminal epithelial cells and in the peripherial stroma. After the blastocyst attaches, the luminal epithelium and peripherial stroma at the implantation site strongly express VEGF, with increasing expression on both the mesometrial and antimesometrial poles (Chakraborty et al. 1995). More recent evidence suggests that in the mouse uterus, the VEGF164 is the dominant isoform in mouse decidualization and that regulation of VEGF2-2 shows an appropriate spatiotemporal regulation to transduce VEGF signaling in the decidual response (Halder et al. 2000). In the rabbit, VEGF is highly expressed in the implantation stages with a corresponding induction in VEGF2-2 (Das et al. 1997a). Similar results were found in the pig endometrium showing VEGF and its receptors highly expressed in the implantation stages and under control of ovarian steroid hormones (Welter et al. 2003). The expression of VEGF and its receptors are also coordinated during implantation in the marmoset and macaque, suggesting an instrumental role in primate implantation (Rowe et al. 2003, Wang et al. 2003).

The expression of VEGF-A and its receptors has been well characterized in the human uterus. Several studies have shown that VEGF is expressed throughout the human cycle with the highest expression in glandular epithelium at the secretory phase (Shifren et al. 1996, Torry et al. 1996) and that E2 treatment increases levels of the VEGF121, VEGF165 and VEGF189 isoforms (Bausero et al. 1998). In addition, in the endometrial struma, E2 when combined with P4 induces expression of VEGF189 specifically, suggesting that VEGF189 may have an important role in the human uterus (Ancelin et al. 2002). VEGF-A levels have also been found to be correlated with several uterine disease states that negatively impact fertility. In women with moderate to severe endometriosis, peritoneal fluid concentrations of VEGF-A were significantly higher in controls (Shifren et al. 1996). Also, the presence of VEGF-A in the majority of uterine leiomyoma suggests that this factor is important for the local angiogenesis and growth of these tumors (Gentry et al. 2001). Decreased cytoblastic expression of VEGF-A and VEGFR-1 have also been associated with increased incidence of severe pre-eclampsia (Zhou et al. 2002).

**Cytokines**

**Leukemia inhibitory factor (LIF)**

LIF is a small heavily glycosylated protein of 180 amino acid residues transcribed from a single gene with high sequence homology conserved between species. (Hilton 1998). It signals through the LIF receptor (LIF-R), which upon binding LIF heterodimerizes with glycoprotein 130 (gp130) leading to signal transduction. LIF functions to modulate differentiation and proliferation.

The biological function of LIF in the uterus has mainly been characterized using mouse models. LIF undergoes a biphasic expression change in early pregnancy. On day 1 of pregnancy, LIF is expressed in the glandular epithelium of the endometrium, with declining expression until day 3. On the morning of day 4, LIF is again strongly expressed in the glandular epithelial cells then with the onset of blastocyst attachment at midnight, the expression of glandular LIF disappears. LIF expression can then be seen in uterine epithelial and stromal cells surrounding the implanting blastocyst. This differential expression of LIF reinforces the necessity of LIF in different stages of implantation (Bhatt et al. 1991, Song et al. 2000). Other animal models (rabbit, pig, cow, sheep, and non-human primates) show similar biphasic regulation (Yang et al. 1995, Vogia-gis et al. 1997, Kholkute et al. 2000, Modric et al. 2000, Oshima et al. 2003). Targeted disruption of the LIF gene confirms that LIF is a necessary maternal factor for implantation. Although LIF null females are viable and ovulate normally, blastocysts fail to implant (Stewart et al. 1992). In contrast, LIF-R null embryos are able to implant indicating that maternal LIF can act through alternative pathways. LIF-R null animals have disrupted placental development and exhibit perinatal lethality (Dani et al. 1998). Since LIF is also expressed in pseudopregnant females, it suggests that its expression is independent of the trophoblast solely under maternal control (Bhatt et al. 1991).

In the human endometrium, LIF expression varies with the menstrual cycle. LIF can be detected at both the mRNA and protein level in the early secretory phase, peaking at the late secretory phase with abundant expression in the luminal and glandular epithelium, the greatest increase occurring during the implantation
during the window of implantation (Laird et al. 1994, Cullinan et al. 1996). Further clinical studies have elucidated a causal relationship between LIF and human infertility. Women with previously unexplained infertility were found to have point mutations in functionally important regions of LIF (Giess et al. 1999). Although subsequent studies have shown point mutations on LIF to be rare (Steck et al. 1999), other evidence shows a statistically significant percentage of women with unexplained fertility have reduced levels of LIF compared with control counterparts in uterine flushes from women with unexplained infertility. Women with pre-implantation defects in human embryos demonstrate a pattern of LIF expression (Laird et al. 1997).

Interleukin 11 (IL-11)

IL-11 is a cytokine with pleiotropic actions with anti-inflammatory activity (Sands et al. 1999), as well as mucosal protective effects (Keith et al. 2004). In the mouse and human uterus, IL-11 signals through one of its cognate receptors, IL-11Ra, which upon binding IL-11 heterodimerizes with glycoprotein 130 (gp130) in the same manner as LIF. The expression of IL-11 has been characterized in the mouse, rat and human, with the biological function characterized primarily in the mouse. In the mouse, IL-11 expression peaks at the time of decidualization, 5.5–7.5 dpc, with strong expression in the developing decidual cells, and is relatively low in the cycling uterus. The concentration of IL-11Ra and gp130 show no change in expression (Robb et al. 1998). Targeted disruption of the IL-11 locus confirms the importance of maternal IL-11 for the decidual response (Robb et al. 1998). Further studies utilizing a hypomorphic allele of IL-11 showed that only underdeveloped deciduas degenerated before placentation formation in response to implantation (Bilinski et al. 1998).

Several studies have been performed on the expression of IL-11 in human endometrium. Studies consistently show that IL-11 and IL-11Ra mRNA are constant throughout the menstrual cycle, and highly expressed in the glandular epithelium. However, there are discrepancies in whether IL-11 stromal expression remains constant or is upregulated in the secretory phase (Dimitriadis et al. 2000, Cork et al. 2002, Karpovich, Chobotova et al. 2003).

Colony stimulating factor-1 (CSF-1)

CSF-1, or macrophage CSF (m-CSF), is a glycosylated homodimer with essential disulfide-linkages that has a molecular size of 47–76 kDa (Das & Stanley 1982). The human CSF-1 gene is alternatively spliced leading to three major transcripts, all with biological activity (Ladner et al. 1987). CSF-1 modulates differentiation, survival and proliferation in numerous cell types (Stanley et al. 1983). CSF-1 increases approximately 1000 fold during pregnancy with a concomitant increase in CSF-1 mRNA in the luminal and glandular epithelium (Pollard et al. 1987, Arceci et al. 1992). In mice, CSF-1 is first expressed 3 dpc, and increases throughout pregnancy, reaching a peak at 14–15 dpc (Arceci et al. 1989). CSF-1 expression is similar in the pig, where expression is seen throughout gestation with the major increase occurring between days 20 and 30, after which time, high levels of mRNA were maintained to term (Tuo et al. 1995). Cattle also have a similar pattern of expression (Lee et al. 2003).

The biological significance of CSF-1 in the uterus was first realized in the osteopetrotic (op/op) mice, in which a frameshift mutation forms a functional knockout of the CSF-1 gene (Pollard et al. 1991). Mice with the op/op allele have numerous reproductive abnormalities. Females have a low ovulation rate, and decreased implantation and fetal survival rates. Treatment of op/op mice with human recombinant CSF-1 fails to rescue the reproductive phenotype, suggesting that local synthesis of CSF-1 is necessary for uterine function (Wiktor-Jedrzejczak et al. 1991). In addition CSF-1 accelerates the formation of the blastocyst cavity and proliferation of the trophoblast lineages (Pollard 1997).

CSF-1 is expressed in the human endometrial tissue during normal cycling, with higher levels during the luteal phase than during the proliferative phase. In addition, first trimester decidual tissue has higher levels of CSF-1 than non-pregnant controls (Kauma et al. 2001). In addition to the expression of CSF-1 in human pregnancy, several correlative links have been made between abnormal CSF-1 expression and infertility. Low circulating levels of CSF-1 in both preconceptional and 8 week gestational stages are associated with recurrent spontaneous abortion (Katano et al. 1997). Additionally, a comparison between CSF-1 levels in follicular fluid and blood plasma that included fertile and infertile women demonstrated an elevation in the follicular fluid/plasma ratio of CSF-1 levels in the infertile group (Shinetugs et al. 1999).

Cyclooxygenase-2 (Cox-2)

In the uterus, prostaglandins (PGs) are involved in implantation, control of cytokine release, cell growth and differentiation and vascular responses (reviewed in Kelly et al. 2001). Cox proteins are responsible for the rate limiting step in PG synthesis. They convert arachidonic acid into intermediate endoperoxidases (PGH2) which are then converted into PGs by specific synthetases synthases. There are two isoforms of Cox, Cox-1 and Cox-2, that are encoded by separate genes. In general, Cox-1 is a constitutively active gene necessary for routine cellular functions. Cox-2, on the other hand, is induced by a number of different stimuli (Smith & Dewitt 1996). In the mouse, Cox-2 expression has been correlated with blastocyst attachment, as expression is observed in the luminal epithelium and subepithelial stroma at the site of attachment. Gene targeting experiments have demonstrated the necessity of Cox-2 in female reproductive function. In specific mouse lines, Cox-2 null females have numerous reproductive abnormalities including defects in ovulation, fertilization and implantation (Lim et al. 1997). However, in other...
mouse lines, Cox-1 expression can partially compensate for Cox-2 (Wang et al. 2004). Other studies have shown that Cox-2 derived PGs affect activation of peroxisome proliferators-activated receptor delta (PPARδ). PPARδ heterodimerizes with retinoid X receptors (RXRs) to transcriptionally regulate genes (Lim et al. 1999a). In addition, the attenuation of angiogenesis in Cox-2 null mice may be due to a lack of VEGF and VEGFR-2 induction, but normal expression of angiopoietins (Matsumoto et al. 2002a). To date, expression of Cox-2 in the receptive uterus seems to be conserved across all species examined, including guinea pigs (Bracken et al. 1997), sheep (Chargipny et al. 1997), horses (Boerboom et al. 2004), rhesus monkeys (Sun et al. 2004), and baboons (Strakova et al. 2002).

In the human, during the luteal phase, expression of Cox-2 is found in the luminal epithelium and the perivascular cells (Marions & Danielsson 1999). In addition, several disorders that lead to impaired fertility showed a misregulation of Cox-2. Numerous studies have shown that Cox-2 is highly expressed in endometriosis (Ota et al. 2001, Chishima et al. 2002). Other studies show that Cox-2 is strongly expressed in endometrial adenocarcinomas (Uotila et al. 2002), but not in preeclampsia (Wetzka et al. 1997).

**Modulators of cell adhesion**

**Mucin 1 (Muc1)**

Muc1, a negative regulator of cellular adhesion, is a heavily glycosylated, high molecular weight membrane protein whose large extracellular domain consists of a variable number of tandem repeats of 20 conserved amino acids, each containing five potential O-linked glycosylation sites (Gendler et al. 1990). The variable number of these tandem repeats (16 in mice, 20–125 in humans) leads to substantial polymorphic variation in the final gene product (Horne et al. 2001). These tandem repeats seem to sterically hinder adhesion-promoting molecules and have the net effect of inhibiting cell–cell adhesion.

In the uterus, Muc1 is restricted to the epithelium, and high expression of Muc1 is inhibitory to blastocyst attachment in all species studied thus far. In addition, there has been numerous reports that show that Muc1 is regulated by steroid hormones across species. In mice, Muc1 expression is stimulated by E2 and repressed by P4 (Surveyor et al. 1995). Regulation of Muc1 expression in pigs is similar to mice (Bowen et al. 1996) while in baboons and rabbits it is by P4 only (Bowen et al. 1996), in baboons and rabbits, by P4 only (Hild-Petito et al. 1996, Hoffman et al. 1998). However, the expression pattern of Muc1 in the endometrium shows species to species variation. In mice, Muc1 is greatly reduced in luminal epithelium during the window of implantation. However, in both humans and rabbits, expression of Muc1 is increased during the receptive phase (Hey et al. 1994, Hoffman et al. 1998), but in vitro studies have shown that during the adhesion phase of implantation, the blastocyst induces a paracrine signal to cleave the Muc1 extracellular domains at the site of attachment (Hoffman et al. 1998, Meseguer et al. 2001). Although mice null for Muc1 are fertile when isolated in pathogen free environments, under normal housing conditions, chronic infection and inflammation of the lower reproductive tract occurs due to opportunistic infection by common flora. These chronic infections then lead to reduced fertility rates showing the necessity of MUC1 in natural environments (DeSouza et al. 1999).

In the human uterus, studies have correlated Muc1 levels with fertility. The concentrations of Muc1 proteins in uterine flushings in women suffering from recurrent spontaneous miscarriages was significantly lower than in the controls throughout the luteal phase and most drastically at the time of implantation (Hey et al. 1995). In addition, the polymorphic variation due to the number of tandem repeats that range from 20–125 in humans has also been implicated in infertility. Women with unexplained infertility have a median allele size that is significantly smaller than control groups (Horne et al. 2001).

**Integrins**

Integrins are transmembrane glycoproteins composed of an α- and a β-subunit that are noncovalently associated (Alberts et al. 1994). These heterodimers comprise a major class of cell adhesion molecules. Both constitutive and cyclical expression of integrins in the uterus has been observed, and they are now considered the most decisive criterion for determining uterine receptivity (Lessey et al. 1992, 1994, 1996). Although there are many integrin heterodimer pairs expressed in the human uterus, the apical localization of αvβ3 and αvβ5 integrins in the mouse, human, baboon, rabbit, pig, and sheep luminal epithelium, makes these specific integrin pairs appropriate candidates for mediating trophoblast/epithelial interactions (Bowen et al. 1996, Lessey et al. 1996, Fazleabas et al. 1997, Burghardt et al. 2002, Illera et al. 2003). Furthermore, in the human baboon, and pig, integrin β3 is temporally regulated, such that its expression coincides with the window of receptivity (Lessey et al. 1992, Bowen et al. 1996, Fazleabas et al. 1997). Although no cyclical regulation of these integrins is seen in mice, it is possible that these integrins are masked by Muc1 in the prereceptive phase (Wesseling et al. 1995). Recent evidence reveals that αvβ3, in cows and shee, does not have a similar expression pattern to other species examined (Kimmis et al. 2004).

The blastocyst also expresses several integrins. In early blastocyst development α5β1 and α6β1 integrins are expressed on the cavity of the trophoectoderm cells and cells that make up the ICM (Sutherland et al. 1993). However, as the blastocyst becomes active, the integrins translocate to the apical surface of the trophoectoderm, implying a possible role in initial attachment after hatching from the zona pellucida (Schultz et al. 1997). The integrin αvβ3 has also been shown on the surface of the blastocyst (Sutherland et al. 1993). Since the
blastocyst and the luminal epithelium both contain integrins on their respective apical surfaces, a reciprocal and cooperative role in attachment is suggested. Relevant ligands for the integrins αvβ3 and αvβ5 are known to recognize an Arg-Gly-Asp (RGD) sequence (Armand et al. 1986). Possible ligands for the integrins on the maternal surface include osteopontin, laminin, thrombospondin, and perlecans (Kimber & Spanwick 2000), and on the blastocyst, the oncofetal fibronectin, a protein restricted only to trophoblasts (Feinberg et al. 1991, Craven et al. 2000). In addition, in vitro studies have shown that αvβ3 may also interact and activate specific matrix metalloproteinases in the extracellular matrix of the uterus (Xu et al. 2001), thereby implicating integrins in the transition between attachment and epithelial cell penetration.

The biological function of integrin αvβ3 in mouse implantation has been demonstrated by intratuterine injections of RGD-containing peptides, to out-compete endogenous targets, and antibodies specific to αvβ3. Mice treated in this manner had significantly reduced implantation (Ilhura et al. 2000). However, mice made with a null mutation of integrin β3 showed a mild placentaion defect and no implantation defect, suggesting that there may be redundant function between the integrins in mice (McHugh et al. 2000). In humans, several disorders that lead to impaired fertility showed a misregulation of integrin β3. In a study of women with unexplained infertility, a statistically significant number of women had a significantly reduced expression of integrin β3 compared with fertile controls (Lessey et al. 1995). Although contradictory reports have been made, women with endometriosis have altered β3 expression either in the glandular epithelium or the endometrial vasculature (Lessey et al. 1994, Lessey & Castelbaum 1998). Finally, women with hydrosalpinges, an accumulation of fluid within the fallopian tube, showed reduced β3 levels (Meyer et al. 1997).

**Basigin (Bsg)**

Bsg is a transmembrane glycoprotein with 2 immuno-lobulin domains and a molecular weight between 43 and 66 kDa (Mizukami et al. 1991) that has potential roles in cell–cell communication. Although little is known about Bsg function in the avian and mammalian uterus, null mutations in the mouse have shown that Bsg is a necessary gene in implantation (Kuno et al. 1998). Mice null for Bsg generally die either in utero or within a month after birth from interstitial pneumonia. The null mice that do reach maturity exhibit both male and female reproductive phenotypes (Igakura et al. 1998). Although the female mice are able to cycle and mate normally, multiple reproductive failures are evident, including fertilization and implantation. Embryo transfer experiments into the uterus of Bsg null females demonstrate that Bsg is necessary for implantation (Kuno et al. 1998). In the mouse and rat, Bsg is an E2-regulated gene with decreasing expression in the uterine epithelial tissues on days 1–4. Conversely, in the stroma, expression is first detected on day 3, and increases until implantation. At the time of implantation, Bsg is found in the embryo, and the luminal epithelium and peri-epithelial stroma around the implantation site. In accordance with this expression pattern, Bsg is induced by artificial decidualization (Xiao et al. 2002a,b). In the rabbit, the Bsg homologue, GP42 or haptoglobin (Schuster et al. 1996), is also found to be expressed at the time of implantation (Hoffman et al. 1996, Olson et al. 1997). Although no correlations between Bsg and human implantation have been published, like integrin αvβ3, in cell culture Bsg has also been shown to activate matrix metalloproteinases elucidating a possible role in epithelial cell penetration (Biswas et al. 1995).

**Developmental factors**

**Homeobox (Hox) Genes**

Hox genes were first described in *Drosophila melanogaster* as genes that are important for establishing segment identity during development. Mutation or misexpression of Hox genes leads to the development of one body segment in place of another (McGinnis et al. 1984). When Hox genes are ablated in the fruit fly, the body segment in which it is normally expressed develops the characteristics of an adjacent segment, usually anterior, but occasionally posterior (Hunt et al. 1991). This phenomenon is known as anterior or posterior transformation. Hox genes are characterized by a well-conserved 183 base pair region that encodes a homeodomain which binds DNA through a helix–loop–helix motif to alter gene transcription (Affolter et al. 1991, McGinnis & Krumlauf 1992). To date, all research into the role of Hox genes in uterine development and implantation has occurred solely in mice and humans.

Taylor et al. (1997) characterizes the Hox genes and the expression patterns necessary for the development of the mouse reproductive tract. These genes are *Hoxa9, Hoxa10, Hoxa11*, and *Hoxa13*. In Mullerian tract development before differentiation into the fallopian tube, uterus, cervix, and vagina, all four Hox genes are found throughout the tube. However, during structural differentiation that occurs from birth to 2 weeks, the expression pattern of the Hox genes becomes restricted and leads to the development of distinct structures. *Hoxa9* is expressed in the presumptive fallopian tube, *Hoxa10* is expressed in the developing uterus, *Hoxa11* is in the posterior uterine segment and the cervix, and *Hoxa13* is found in the primordial vagina (Taylor et al. 1997). In the adult mouse uterus, both *Hoxa10* and *Hoxa11* are regulated by P4 acting through its cognate receptor, PR (Ma et al. 1998). This data corresponds to previous data showing that in *Hoxa10* null mice, only P4-dependent stromal, and not E2-dependent epithelial, cell proliferation is abrogated (Lim et al. 1999b). Gene targeting experiments to ablate specific Hox genes have elucidated their developmental and reproductive function. Mice null for either *Hoxa10* or
Hoxa11 show implantation failure (Hunt et al. 1991, Satokata et al. 1995). However, this phenotype is confounded by subtle developmental phenotypes. The anterior 25% of Hoxa10 null mice uterus undergo anterior transformation into an oviduct like structure (Benson et al. 1996), and the Hoxa11 null mice exhibit decreased stromal development and expression of LIF (Gendron et al. 1997). To eliminate developmental effects, intratraheine transfection of antisense oligonucleotides to Hoxa10 into the uterine lumen on day 2 of pregnancy was undertaken. This treatment greatly reduced the number of implantation sites, thus supporting the role of adult maternal Hox proteins for implantation (Bagot et al. 2000). Additionally, the ablation of another Hox gene, Hmx3, has also been shown to be important for implantation in mice. Although an exploration of its affect on infertility has yet to be done, a perturbation of the Wnt and LIF gene expression in the female null uterus may be contributing factors (Wang et al. 1998).

Hoxa10 and Hoxa11 have also been implicated to have a role in human reproduction. Cell culture experiments have shown that expression of both Hox genes are regulated by E2 and P4, and that they are expressed in the glandular epithelia and the stroma of human uterus (Taylor et al. 1998, 1999). Although Hox expression occurs throughout the menstrual cycle, there is a significant increase in expression during the mid and late luteal phases and the time of implantation (Taylor et al. 1998, 1999). In patients with endometriosis, there is no mid-luteal increase of HoxA10 and Hoxa11 (Taylor et al. 1999), and similar patterns are seen for HoxA10 in patients diagnosed with leiomyomas (Cermik et al. 2002).

Wnt Genes

Wnt genes are vertebrate homologs of the Drosophila melanogaster segment polarity gene wingless. Wnt proteins form a family of highly conserved secreted glycoproteins that are critical for cell–cell communication, cell fate specification, growth, and differentiation during development of vertebrates and invertebrates (Wodarz & Nusse 1998, Yamaguchi 2001). Frizzled proteins, characterized by a seven-pass transmembrane region with cystine rich extracellular domains, act as the Wnt receptors (Bhanot et al. 1996). The binding of Wnts to these receptors activates Disheveled, a cytoplasmic phosphoprotein whose activation leads to a complex signal transduction cascade resulting in the activation of β-catenin (Ikeda et al. 1998). To date, most of the published research on the expression and function of Wnts in the uterus has been performed in the mouse and human.

Miller et al. (1998) described differential expression patterns of Wnt genes throughout development and estrous cycle in mice. Three Wnt genes, Wnt4, Wnt5a and Wnt7a, are highly expressed in the developing and adult uterus. During development, Wnt4 is expressed strongly in the subepithelial mesenchyme with no expression in the luminal epithelium. In the adult animal, Wnt4 expression fluctuates with the estrous cycle with the peak of expression at estrus in the luminal epithelium and stroma. During implantation, Wnt4 is strongly induced in the primary decidual region with expression expanding to the secondary decidual zone (Paria et al. 2001, Daikoku et al. 2004). Wnt5a is expressed in the mesenchyme during development with primarily stromal expression in the adult animal. Wnt7a is expressed in the luminal epithelium, as well as, the glands during development. In the adult, the expression of Wnt7a in the glandular epithelium is lost, and Wnt7a is solely expressed in the luminal epithelium with minor fluctuations during the estrous cycle (Miller et al. 1998). Knockout mice for all three of these Wnt genes have been generated and demonstrate the necessity of these genes in the developing uterus. Wnt4 null females die at birth and show a complete failure of Müllerian duct formation (Vainio et al. 1999). Wnt5a null females also die at birth (Yamaguchi et al. 1999). However, tissue grafting experiments of embryonic female reproductive tracts demonstrate the importance of Wnt5a. In these experiments, Wnt5a null uterus show numerous developmental failures. The uterine are shorter, with thinner myometrial layers, and have an absence or dramatic reduction in uterine gland formation (Mericckay et al. 2004). Unlike Wnt4 and Wnt5a null animals, Wnt7a null females are viable, however, they also display a severe, abnormal developmental phenotype that is suggestive of complete posterior transformation of the female reproductive tract. Wnt7a null ovaries acquire cellular and molecular characteristics of the uterus, while the uterus exhibits an intermediate phenotype with characteristics of both the uterus and vagina, as well as, a complete lack of glandular formation (Miller & Sassoon 1998).

The complexity of these phenotypes is due in part to the extensive regulatory loops between the individual Wnt proteins as well as the Hox proteins and other implantation related factors. In response to E2 treatment, Wnt5a has regulatory effects on Wnt7a expression (Mericckay et al. 2004). Wnt7a null females lose Hoxa-10 and Hoxa-11 expression leading to posterior transformation (Miller & Sassoon 1998). In turn, in the adult uterus, Hoxa-10 mutants show aberrant expression of Wnt4 (Daikoku et al. 2004), and Hoxa-11 null mice display a loss of LIF expression (Gendron et al. 1997). Therefore, there is clearly an intricate and essential signaling cascade between these developmental factors, cytokines, and the steroid hormones that is involved in uterine gene regulation.

Although Wnt7a mutations are unlikely to be a cause of developmental mutations of the female reproductive tract in humans (Timmreck et al. 2003), an inverse correlation between ER-α and Wnt7a expression levels has been found in leiomyomas. It is possible that hypersensitivity to E2 deregulates Wnt7a expression resulting in loss of myometrial patterning and development of leiomyomas (Li et al. 2001). In the cycling human endometrium, the
expression of several Wnts and members of the Wnt signal transduction cascade were analyzed. Although no significant change was found in most of the Wnt proteins, including Wnt-4, Wnt-5a, Wnt-7a during the menstrual cycle in women, there was a large change in the level of inhibitors of Wnt signaling between the proliferative and secretory phases. This regulation of signaling molecules suggests that the Wnt signaling cascade is conserved between species (Tulac et al. 2003).

Conclusions

In conclusion, the use of animal models in the study of implantation has important ramifications in understanding trophoblast–uterine interactions in the human. The four stages that comprise early implantation in mammals: apposition and adhesion of the blastocyst to the uterine lumen, penetration of the epithelium and decidualization of the stromal cells, and trophoblastic invasion into the stromal vasculature. The physiological mechanisms of each of these stages can be more easily observed and understood in different animal models. A prolonged period of apposition and attachment in pigs and sheep, without invasion and decidualization, makes them outstanding candidate models for studying these early phases (Gray et al. 2001). In addition, the UGKO ewe model can facilitate molecular understanding of these events. The rabbit can also serve as a good candidate model for understanding blastocyst adhesion due to the precise timing of attachment and extreme apical association of the trophoblastic knobs with the luminal epithelium (Hoffman et al. 1998). The direct stromal invasion of the guinea pig and the repeated penetration of the marmoset and rhesus monkey blastocyst make these good models for studying epithelial cell penetration (Enders 2000). Decidualization is extremely straightforward to study in the mouse, as the blastocyst induces the decidual response prior to epithelial penetration (Carson et al. 2000). In addition, in the mouse, the decidualization reaction can be elucidated by trauma or beads soaked in appropriate growth factors (as reviewed in Paria et al. 2001, Dey et al. 2004). Due to easily visible structures and the ability to visualize implantation sites through ultrasonographic techniques, the macaque is a strong candidate animal model for studying late implantation events, including invasion of trophoblast into the maternal blood supply (Enders 2000).

Although the physiological events of implantation are best investigated through a variety of animal models, the in vivo molecular events of implantation, thus far, have largely been described in the mouse. Gene ablation studies have been instrumental in understanding uterine gene function, however, a number of the genes implicated in implantation (Halder et al. 2000, Paria et al. 2001, Daikoku et al. 2003) including IHH, Bone morphogenetic protein 2 (BMP-2), VEGF, Hypoxia Induced Factor 1 (HIF-1), and Wnt4 are lethal at early developmental stages due to perturbation of vascular development, bone morphogenesis, or axis formation (Ferrara et al. 1996, Zhang & Bradley 1996, Iyer et al. 1998, St-Jacques et al. 1999, Vainio et al. 1999). In order to study the function of these genes in the adult uterus, it will be necessary to create models that ablate gene expression in a tissue specific manner. Since there are numerous preexisting lines of mice in which genes have been genetically floxed (http://www.mshri.on.ca/nagy/floxed.html), a uterine specific Cre recombinase would make it possible to ablate genes in the uterus. At this time, a uterine specific Cre recombinase has yet to be developed. However, a mouse model in which the endogenous P4 receptor promoter directly regulates a lacZ reporter demonstrates that genes knocked into the PR locus have high levels of expression throughout the epithelial, stromal and myometrial layers of the uterus (Ismail et al. 2002). Knocking Cre recombinase into the PR locus in a similar method to the lacZ reporter should allow the specific ablation of genes in tissues that express PR, including the postnatal uterus. Potential problems with this mouse model would include disruption of gene expression and function in the pituitary and ovary, as both of these tissues express PR. By creating gene targeted mouse models in the endometrium, the potential exists to eliminate problems associated with early lethality, as well as, avoiding the contradictory results that epigenetic treatments often have. Intraluminal infusions or blastocyst treatment with oligodeoxynucleotides, antibodies, or chemical antagonists designed to block specific proteins often lead to results that are incompatible to the null mutations of genes. Some examples of this are the integrin β3 (mentioned earlier), AR, (Tsark et al. 1997, Luetteke et al. 1999), calcitonin (Zhu et al. 1998, Hoff et al. 2002), and IL-1 receptor (Abbondanzo et al. 1996, Simon et al. 1998). The apparently contradictory results might be due to lack of specificity of the treatment, or may simply not account for compensatory mechanisms developed in the null animals.

The use of animal models to study implantation has great importance. The advent of cDNA microarray technology has begun to elucidate the global gene changes that allow implantation in the mouse and the human, and gene targeting experiments in the mouse have resolved the uterine function of many genes. However, much work still remains to identify factors that are conserved between species. Additionally, the variations in timing and mechanisms of the animal models will help to elucidate the function of these genes. As can be seen from the well-characterized factors mentioned above, these models can facilitate identification of factors necessary in human implantation.

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