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.

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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 estrone (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 syncytial trophoblast 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 trophoblast 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 epitheliochorial placental 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 caruncles, stromal aglandular 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 trophoblast 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 epitheliochorial, and these models serve as further 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, blastocysts 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 syncytial 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 syncytial 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 syncytial 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 previllous stage follows and is characterized by the formation of connected syncytial trophoblast-lined lacunae that eventually become filled with maternal blood (Enders 2000). Studies in the macaque show that the syncytial 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 syncytial 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 transmitting 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 to support 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 isoforms 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-norprogesterone 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 led 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.* 2002), 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 trophoctoderm (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 (Yue *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 (Luetteke *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 trophoctoderm in periimplantation 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, vascularization 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 periepithelial stroma. After the blastocyst attaches, the luminal epithelium and periepithelial 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 VEGFR-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 VEGFR-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 stroma, 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 cytotlastic 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, Vogiatzis *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

window. In the proliferative phase, LIF is undetectable (Charnock-Jones *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.* 2004), other evidence shows a statistically significant percentage of women with unexplained fertility have reduced levels of LIF compared with control counterparts in uterine flushes during the window of implantation (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.* 1994). 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 placental 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.* 1991). 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 (PPAR δ). PPAR δ 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 (Charpigny *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 it 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 α v β 3 and α v β 5 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 β 3 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 α v β 3, in cows and sheep, does not have a similar expression pattern to other species examined (Kimmins *et al.* 2004).

The blastocyst also expresses several integrins. In early blastocyst development α 5 β 1 and α 6 β 1 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 α v β 3 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 $\alpha v\beta 3$ and $\alpha v\beta 5$ are known to recognize an Arg-Gly-Asp (RGD) sequence (Armant *et al.* 1986). Possible ligands for the integrins on the maternal surface include osteopontin, laminin, thrombospondin, and perlecan (Kimber & Spanswick 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 $\alpha v\beta 3$ 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 $\alpha \beta 3$ in mouse implantation has been demonstrated by intrauterine injections of RGD-containing peptides, to out-compete endogenous targets, and antibodies specific to $\alpha v\beta 3$. Mice treated in this manner had significantly reduced implantation (Illera *et al.* 2000). However, mice made with a null mutation of integrin $\beta 3$ showed a mild placenta 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 $\beta 3$. In a study of women with unexplained infertility, a statistically significant number of women had a significantly reduced expression of integrin $\beta 3$ compared with fertile controls (Lessey *et al.* 1995). Although contradictory reports have been made, women with endometriosis have altered $\beta 3$ 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 $\beta 3$ levels (Meyer *et al.* 1997).

Basigin (Bsg)

Bsg is a transmembrane glycoprotein with 2 immunoglobulin 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 $\alpha v\beta 3$, 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 Müllerian tract development before differentiation into the fallopian tube, uterus, cervix, and vagina, all four Hoxa 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 uteri 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, intrauterine 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 uteri (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 estrous 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 uteri show numerous developmental failures. The uteri are shorter, with thinner myometrial layers, and have an absence or dramatic reduction in uterine gland formation (Mericskay *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 oviducts 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 (Mericskay *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 $\beta 3$ (mentioned earlier), AR, (Tsark *et al.* 1997, Luetkeke *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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References

- Abbondanzo SJ, Cullinan EB, McIntyre K, Labow MA & Stewart CL 1996 Reproduction in mice lacking a functional type 1 IL-1 receptor. *Endocrinology* **137** 3598–3601.
- Adams EC, Hertig AT & Rock J 1956 A description of 34 human ova within the first 17 days of development. *American Journal of Anatomy* **98** 435–493.
- Affolter M, Percival-Smith A, Muller M, Billeter M, Qian YQ, Otting G, Wuthrich K & Gehring WJ 1991 Similarities between the homeodomain and the Hin recombinase DNA-binding domain. *Cell* **64** 879–880.
- Alberts B, Bray D, Lewis J, Raff M, Roberts K & Watson JD 1994 *Molecular Biology of the Cell*, 3rd edn. New York: Garland Publishing Inc. p 996.
- Albrecht ED, Aberdeen GW, Niklaus AL, Babischkin JS, Suresch DL & Pepe GJ 2003 Acute temporal regulation of vascular endothelial growth/permeability factor expression and endothelial morphology in the baboon endometrium by ovarian steroids. *Journal of Clinical Endocrinology and Metabolism* **88** 2844–2852.
- Ancelin M, Buteau-Lozano H, Meduri G, Osborne-Pellegrin M, Sordello S, Plouet J & Perrot-Applanat M 2002 A dynamic shift of VEGF isoforms with a transient and selective P4-induced expression of VEGF189 regulates angiogenesis and vascular permeability in human uterus. *PNAS* **99** 6023–6028.
- Arceci RJ, Shanahan F, Stanley ER & Pollard JW 1989 Temporal expression and location of colony-stimulating factor 1 (CSF-1) and its receptor in the female reproductive tract are consistent with CSF-1-regulated placental development. *PNAS* **86** 8818–8822.
- Arceci RJ, Pampfer S & Pollard JW 1992 Expression of CSF-1/c-fms and SF/c-kit mRNA during preimplantation mouse development. *Developmental Biology* **151** 1–8.
- Armant DR, Kaplan HA, Mover H & Lennarz WJ 1986 The effect of hexapeptides on attachment and outgrowth of mouse blastocysts cultured *in vitro*: evidence for the involvement of the cell recognition tripeptide Arg-Gly-Asp. *PNAS* **83** 6751–6755.
- Bagot CN, Troy PJ & Taylor HS 2000 Alteration of maternal Hoxa10 expression by *in vivo* gene transfection affects implantation. *Gene Therapy* **7** 1378–1384.
- Bartol FF, Wiley AA, Coleman DA, Wolfe DF & Riddell MG 1988 Ovine uterine morphogenesis: effects of age and progestin administration and withdrawal on neonatal endometrial development and DNA synthesis. *Journal of Animal Science* **66** 3000–3009.
- Bausero P, Cavaille F, Meduri G, Freitas S & Perrot-Applanat M 1998 Paracrine action of vascular endothelial growth factor in the human endometrium: production and target sites, and hormonal regulation. *Angiogenesis* **2** 167–182.
- Bazer FW 1975 Uterine protein secretions: Relationship to development of the conceptus. *Journal of Animal Science* **41** 1376–1382.
- Benson GV, Lim H, Paria BC, Satokata I, Dey SK & Maas RL 1996 Mechanisms of reduced fertility in Hoxa-10 mutant mice: uterine homeosis and loss of maternal Hoxa-10 expression. *Development* **122** 2687–2696.
- Bhanot P, Brink M, Samos CH, Hsieh JC, Wang Y, Macke JP, Andrew D, Nathans J & Nusse R 1996 A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. *Nature* **382** 225–230.
- Bhatt H, Brunet LJ & Stewart CL 1991 Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. *PNAS* **88** 11408–11412.
- Bilinski P, Roopenian D & Gossler A 1998 Maternal IL-11R alpha function is required for normal decidua and fetoplacental development in mice. *Genes and Development* **12** 2234–2243.
- Biswas C, Zhang Y, DeCastro R, Guo H, Nakamura T, Kataoka H & Nabeshima K 1995 The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. *Cancer Research* **55** 434–439.
- Boerboom D, Brown KA, Vaillancourt D, Poitras P, Goff AK, Watanabe K, Dore M & Sirois J 2004 Expression of key prostaglandin synthases in equine endometrium during late diestrus and early pregnancy. *Biology of Reproduction* **70** 391–399.
- Bowen JA, Bazer FW & Burghardt RC 1996 Spatial and temporal analyses of integrin and Muc-1 expression in porcine uterine epithelium and trophoblast *in vivo*. *Biology of Reproduction* **55** 1098–1106.
- Bracken KE, Elger W, Jantke I, Nanninga A & Gellersen B 1997 Cloning of guinea pig cyclooxygenase-2 and 15-hydroxyprostaglandin dehydrogenase complementary deoxyribonucleic acids: steroid-modulated gene expression correlates to prostaglandin F2 alpha secretion in cultured endometrial cells. *Endocrinology* **138** 237–247.
- Burghardt RC, Johnson GA, Jaeger LA, Ka H, Garlow JE, Spencer TE & Bazer FW 2002 Integrins and extracellular matrix proteins at the maternal–fetal interface in domestic animals. *Cells Tissues Organs* **172** 202–217.
- Canivenc R, Laffargue M & Mayer G 1956 Nidations retardées chez la ratte castrée et injectée de progesterone: Influence du moment de la castration sur la chronologie de l'ovimplantation. *CR Seances Soc Biol* **150** 2208–2212.
- Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA & Yoshinaga K 2000 Embryo implantation. *Developmental Biology* **223** 217–237.
- Cermik D, Arici A & Taylor HS 2002 Coordinated regulation of HOX gene expression in myometrium and uterine leiomyoma. *Fertility and Sterility* **78** 979–984.
- Chakraborty I, Das SK & Dey SK 1995 Differential expression of vascular endothelial growth factor and its receptor mRNAs in the mouse uterus around the time of implantation. *Journal of Endocrinology* **147** 339–352.
- Chambon Y 1949 Besoins endocriniens qualitatifs et quantitatifs de l'ovimplantation chez la laline. *CR Seance Soc Biol* **143** 1171–1175.
- Charnock-Jones DS, Sharkey AM, Rajput-Williams J, Burch D, Schofield JP, Fountain SA, Boock CA & Smith SK 1993 Identification and localization of alternately spliced mRNAs for vascular endothelial growth factor in human uterus and E2 regulation in endometrial carcinoma cell lines. *Biology of Reproduction* **48** 1120–1128.
- Charnock-Jones DS, Sharkey AM, Fenwick P & Smith SK 1994 Leukaemia inhibitory factor mRNA concentration peaks in human endometrium at the time of implantation and the blastocyst contains mRNA for the receptor at this time. *Journal of Reproduction and Fertility* **101** 421–426.
- Chapigny G, Reinaud P, Tamby JP, Creminon C, Martal J, Maclouf J & Guillomot M 1997 Expression of cyclooxygenase-1 and -2 in ovine endometrium during the estrous cycle and early pregnancy. *Endocrinology* **138** 2163–2171.
- Chishima F, Hayakawa S, Sugita K, Kinukawa N, Aleemuzzaman S, Nemoto N, Yamamoto T & Honda M 2002 Increased expression of cyclooxygenase-2 in local lesions of endometriosis patients. *American Journal of Reproductive Immunology* **48** 50–56.
- Chobotova K, Spyropoulou I, Carver J, Manek S, Heath JK, Gullick WJ, Barlow DH, Sargent IL & Mardon HJ 2002 Heparin-binding epidermal growth factor and its receptor ErbB4 mediate implantation of the human blastocyst. *Mechanisms of Development* **119** 137–144.
- Cork BA, Tuckerman EM, Li TC & Laird SM 2002 Expression of interleukin (IL)-11 receptor by the human endometrium *in vivo* and effects of IL-11, IL-6 and LIF on the production of MMP and cytokines by human endometrial cells *in vitro*. *Molecular Human Reproduction* **8** 841–848.
- Craven CM, Zhao L & Ward K 2000 Lateral placental growth occurs by trophoblast cell invasion of decidual veins. *Placenta* **21** 160–169.
- Cullinan EB, Abbondanzo SJ, Anderson PS, Pollard JW, Lessey BA & Stewart CL 1996 Leukemia inhibitory factor (LIF) and LIF receptor

- expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. *PNAS* **93** 3115–3120.
- Curtis SW, Clark J, Myers P & Korach KS** 1999 Disruption of E2 signaling does not prevent P4 action in the E2 receptor alpha knockout mouse uterus. *PNAS* **96** 3646–3651.
- Curtis Hewitt S, Goulding EH, Eddy EM & Korach KS** 2002 Studies using the E2 receptor alpha knockout uterus demonstrate that implantation but not decidualization-associated signaling is E2 dependent. *Biology of Reproduction* **67** 1268–1277.
- Daikoku T, Matsumoto H, Gupta RA, Das SK, Gassmann M, DuBois RN & Dey SK** 2003 Expression of hypoxia-inducible factors in the peri-implantation mouse uterus is regulated in a cell-specific and ovarian steroid hormone-dependent manner. Evidence for differential function of HIFs during early pregnancy. *Journal of Biological Chemistry* **278** 7683–7691.
- Daikoku T, Song H, Guo Y, Riesewijk A, Mosselman S, Das SK & Dey SK** 2004 Uterine Msx-1 and Wnt4 signaling becomes aberrant in mice with the loss of leukemia inhibitory factor or Hoxa-10: evidence for a novel cytokine-homeobox-Wnt signaling in implantation. *Molecular Endocrinology* **18** 1238–1250.
- Dani C, Chambers I, Johnstone S, Robertson M, Ebrahimi B, Saito M, Taga T, Li M, Burdon T, Nichols J & Smith A** 1998 Paracrine induction of stem cell renewal by LIF-deficient cells: a new ES cell regulatory pathway. *Developmental Biology* **203** 149–162.
- Das SK & Stanley ER** 1982 Structure-function studies of a colony stimulating factor (CSF-1). *Journal of Biological Chemistry* **257** 13679–13684.
- Das SK, Wang XN, Paria BC, Damm D, Abraham JA, Klagsbrun M, Andrews GK & Dey SK** 1994 Heparin-binding EGF-like growth factor gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition: a possible ligand for interaction with blastocyst EGF-receptor in implantation. *Development* **120** 1071–1083.
- Das SK, Chakraborty I, Paria BC, Wang XN, Plowman G & Dey SK** 1995 Amphiregulin is an implantation-specific and P4-regulated gene in the mouse uterus. *Molecular Endocrinology* **9** 691–705.
- Das SK, Chakraborty I, Wang J, Dey SK & Hoffman LH** 1997a Expression of vascular endothelial growth factor (VEGF) and VEGF-receptor messenger ribonucleic acids in the peri-implantation rabbit uterus. *Biology of Reproduction* **56** 1390–1399.
- Das SK, Das N, Wang J, Lim H, Schryver B, Plowman GD & Dey SK** 1997b Expression of betacellulin and epiregulin genes in the mouse uterus temporally by the blastocyst solely at the site of its apposition is coincident with the 'window' of implantation. *Developmental Biology* **190** 178–190.
- Deanesly R** 1960 Normal implantation in ovariectomized guinea pigs. *Nature* **186** 327–328.
- DeSouza MM, Surveyor GA, Price RE, Julian J, Kardon R, Zhou X, Gendler S, Hilkins J & Carson DD** 1999 MUC1/episialin: a critical barrier in the female reproductive tract. *Journal of Reproductive Immunology* **45** 127–158.
- Dey SK** 2003 Visualizing Early Embryo Implantation Sites by Dye Injection. In *Manipulating the Mouse Embryo*, Ed. A Nagy. Cold Spring Harbor, NY: Cold Spring Harbor Press. pp 10–11.
- Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T & Wang H** 2004 Molecular cues to implantation. *Endocrine Reviews* **25** 341–373.
- Dimitriadis E, Salamonsen LA & Robb L** 2000 Expression of interleukin-11 during the human menstrual cycle: coincidence with stromal cell decidualization and relationship to leukaemia inhibitory factor and prolactin. *Molecular Human Reproduction* **6** 907–914.
- Ding YQ, Zhu LJ, Bagchi MK & Bagchi IC** 1994 P4 stimulates calcitonin gene expression in the uterus during implantation. *Endocrinology* **135** 2265–2274.
- Enders AC** 2000 Trophoblast-uterine interactions in the first days of implantation: models for the study of implantation events in the human. *Seminars in Reproductive Medicine* **18** 255–263.
- Enders AC & Schlafke S** 1969 Cytological aspects of trophoblast-uterine interaction in early implantation. *American Journal of Anatomy* **125** 1–29.
- Enders AC & Schlafke S** 1971 Penetration of the uterine epithelium during implantation in the rabbit. *American Journal of Anatomy* **132** 219–230.
- Enders AC & Blankenship TN** 1999 Comparative placental structure. *Advanced Drug Delivery Reviews* **38** 3–15.
- Enders AC & Lopata A** 1999 Implantation in the marmoset monkey: expansion of the early implantation site. *The Anatomical Record* **256** 279–299.
- Enders AC, Hendrickx AG & Schlafke S** 1983 Implantation in the rhesus monkey: initial penetration of endometrium. *American Journal of Anatomy* **167** 275–298.
- Enders AC, Schlafke S & Hendrickx AG** 1986 Differentiation of the embryonic disc, amnion, and yolk sac in the rhesus monkey. *American Journal of Anatomy* **177** 161–185.
- Enders AC, Lantz KC & Schlafke S** 1989 Differentiation of trophoblast of the baboon blastocyst. *The Anatomical Record* **225** 329–340.
- Fazleabas AT, Bell SC, Fleming S, Sun J & Lessey BA** 1997 Distribution of integrins and the extracellular matrix proteins in the baboon endometrium during the menstrual cycle and early pregnancy. *Biology of Reproduction* **56** 348–356.
- Feinberg RF, Kliman HJ & Lockwood CJ** 1991 Is oncofetal fibronectin a trophoblast glue for human implantation? *American Journal of Pathology* **138** 537–543.
- Ferrara N & Davis-Smyth T** 1997 The biology of vascular endothelial growth factor. *Endocrine Reviews* **18** 4–25.
- Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, Hillan KJ & Moore MW** 1996 Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* **380** 439–442.
- Fong GH, Rossant J, Gertsenstein M & Breitman ML** 1995 Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* **376** 66–70.
- Geisert RD & Yelich JV** 1997 Regulation of conceptus development and attachment in pigs. *Journal of Reproduction and Fertility Supplement* **52** 133–149.
- Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, Duhig T, Peat N, Burchell J, Pemberton L, Lalani EN & Wilson D** 1990 Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *Journal of Biological Chemistry* **265** 15286–15293.
- Gendron RL, Paradis H, Hsieh-Li HM, Lee DW, Potter SS & Markoff E** 1997 Abnormal uterine stromal and glandular function associated with maternal reproductive defects in Hoxa-11 null mice. *Biology of Reproduction* **56** 1097–1105.
- Gentry CC, Okolo SO, Fong LF, Crow JC, Maclean AB & Perrett CW** 2001 Quantification of vascular endothelial growth factor-A in leiomyomas and adjacent myometrium. *Clinical Science* **101** 691–695.
- Giess R, Tanasescu I, Steck T & Sendtner M** 1999 Leukaemia inhibitory factor gene mutations in infertile women. *Molecular Human Reproduction* **5** 581–586.
- Gray CA, Bartol FF, Tarleton BJ, Wiley AA, Johnson GA, Bazer FW & Spencer TE** 2001 Developmental biology of uterine glands. *Biology of Reproduction* **65** 1311–1323.
- Gray CA, Burghardt RC, Johnson GA, Bazer FW & Spencer TE** 2002 Evidence that absence of endometrial gland secretions in uterine gland knockout ewes compromises conceptus survival and elongation. *Reproduction* **124** 289–300.
- Halder JB, Zhao X, Soker S, Paria BC, Klagsbrun M, Das SK & Dey SK** 2000 Differential expression of VEGF isoforms and VEGF(164)-specific receptor neuropilin-1 in the mouse uterus suggests a role for VEGF(164) in vascular permeability and angiogenesis during implantation. *Genesis* **26** 213–224.

- Hewitt SC, Deroo BJ, Hansen K, Collins J, Grissom S, Afshari CA & Korach KS 2003 E2 receptor-dependent genomic responses in the uterus mirror the biphasic physiological response to E2. *Molecular Endocrinology* **17** 2070–2083.
- Hey NA, Graham RA, Seif MW & Aplin JD 1994 The polymorphic epithelial mucin MUC1 in human endometrium is regulated with maximal expression in the implantation phase. *Journal of Clinical Endocrinology and Metabolism* **78** 337–342.
- Hey NA, Li TC, Devine PL, Graham RA, Saravolos H & Aplin JD 1995 MUC1 in secretory phase endometrium: expression in precisely dated biopsies and flushings from normal and recurrent miscarriage patients. *Human Reproduction* **10** 2655–2662.
- Hild-Petito S, Fazleabas AT, Julian J & Carson DD 1996 Mucin (Muc-1) expression is differentially regulated in uterine luminal and glandular epithelia of the baboon (*Papio anubis*). *Biology of Reproduction* **54** 939–947.
- Hilton DJ 1998 Leukemia Inhibitory Factor. In *Cytokines*, pp 277–296. Ed. AR Mire-Sluis. New York: Academic Press.
- Hoff AO, Catala-Lehnen P, Thomas PM, Priemel M, Rueger JM, Nasonkin I, Bradley A, Hughes MR, Ordonez N, Cote GJ, Amling M & Gagel RF 2002 Increased bone mass is an unexpected phenotype associated with deletion of the calcitonin gene. *Journal of Clinical Investigation* **110** 1849–1857.
- Hoffman LH, Winfrey VP, Blaeuer GL & Olson GE 1996 A haptoglobin-like glycoprotein is produced by implantation-stage rabbit endometrium. *Biology of Reproduction* **55** 176–184.
- Hoffman LH, Olson GE, Carson DD & Chilton BS 1998 P4 and implanting blastocysts regulate Muc1 expression in rabbit uterine epithelium. *Endocrinology* **139** 266–271.
- Horne AW, White JO, Margara RA, Williams R, Winston RM & Lalani E 2001 MUC 1: a genetic susceptibility to infertility? *Lancet* **357** 1336–1337.
- Huet-Hudson YM, Andrews GK & Dey SK 1989 Cell type-specific localization of c-myc protein in the mouse uterus: modulation by steroid hormones and analysis of the periimplantation period. *Endocrinology* **125** 1683–1690.
- Hunt P, Whiting J, Nonchev S, Sham MH, Marshall H, Graham A, Cook M, Allemann R, Rigby PW & Gulisano M 1991 The branchial Hox code and its implications for gene regulation, patterning of the nervous system and head evolution. *Development* **2** (Suppl) 63–77.
- Igakura T, Kadomatsu K, Kaname T, Muramatsu H, Fan QW, Miyauchi T, Toyama Y, Kuno N, Yuasa S, Takahashi M, Senda T, Taguchi O, Yamamura K, Arimura K & Muramatsu T 1998 A null mutation in basigin, an immunoglobulin superfamily member, indicates its important roles in peri-implantation development and spermatogenesis. *Developmental Biology* **194** 152–165.
- Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S & Kikuchi A 1998 Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3 β and β -catenin and promotes GSK-3 β -dependent phosphorylation of β -catenin. *Embo Journal* **17** 1371–1384.
- Illera MJ, Cullinan E, Gui Y, Yuan L, Beyler SA & Lessey BA 2000 Blockade of the $\alpha(v)\beta(3)$ integrin adversely affects implantation in the mouse. *Biology of Reproduction* **62** 1285–1290.
- Illera MJ, Lorenzo PL, Gui YT, Beyler SA, Apparao KB & Lessey BA 2003 A role for $\alpha v \beta 3$ integrin during implantation in the rabbit model. *Biology of Reproduction* **68** 766–771.
- Ismail PM, Li J, DeMayo FJ, O'Malley BW & Lydon JP 2002 A novel LacZ reporter mouse reveals complex regulation of the P4 receptor promoter during mammary gland development. *Molecular Endocrinology* **16** 2475–2489.
- Iwamoto R, Yamazaki S, Asakura M, Takashima S, Hasuwa H, Miyado K, Adachi S, Kitakaze M, Hashimoto K, Raab G, Nanba D, Higashiyama S, Hori M, Klagsbrun M & Mekada E 2003 Heparin-binding EGF-like growth factor and ErbB signaling is essential for heart function. *PNAS* **100** 3221–3226.
- Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY & Semenza GL 1998 Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1 α . *Genes and Development* **12** 149–162.
- Jones CJ, Enders AC & Fazleabas AT 2001 Early implantation events in the baboon (*Papio anubis*) with special reference to the establishment of anchoring villi. *Placenta* **22** 440–456.
- Karpovich N, Chobotova K, Carver J, Heath JK, Barlow DH & Mardon HJ 2003 Expression and function of interleukin-11 and its receptor α in the human endometrium. *Molecular Human Reproduction* **9** 75–80.
- Katano K, Matsumoto Y, Ogasawara M, Aoyama T, Ozaki Y, Kajiura S & Aoki K 1997 Low serum MCSF levels are associated with unexplained recurrent abortion. *American Journal of Reproductive Immunology* **38** 1–5.
- Kauma SW, Aukerman SL, Eierman D & Turner T 1991 Colony-stimulating factor-1 and c-fms expression in human endometrial tissues and placenta during the menstrual cycle and early pregnancy. *Journal of Clinical Endocrinology and Metabolism* **73** 746–751.
- Keith JC Jr, Albert L, Sonis ST, Pfeiffer CJ & Schaub RG 1994 IL-11, a pleiotropic cytokine: exciting new effects of IL-11 on gastrointestinal mucosal biology. *Stem Cells* **12** (Suppl 1) 79–89; discussion 89–90.
- Kelly RW, King AE & Critchley HO 2001 Cytokine control in human endometrium. *Reproduction* **121** 3–19.
- Kennedy TG, Brown KD & Vaughan TJ 1994 Expression of the genes for the epidermal growth factor receptor and its ligands in porcine oviduct and endometrium. *Biology of Reproduction* **50** 751–756.
- Kholkute SD, Katkam RR, Nandedkar TD & Puri CP 2000 Leukaemia inhibitory factor in the endometrium of the common marmoset *Callithrix jacchus*: localization, expression and hormonal regulation. *Molecular Human Reproduction* **6** 337–343.
- Kim JG, Vallet JL & Christenson RK 2003 Molecular cloning and endometrial expression of porcine amphiregulin. *Molecular Reproduction and Development* **65** 366–372.
- Kimber SJ & Spanswick C 2000 Blastocyst implantation: the adhesion cascade. *Seminars in Cell and Developmental Biology* **11** 77–92.
- Kimmins S, Lim HC & MacLaren LA 2004 Immunohistochemical localization of integrin α V β 3 and osteopontin suggests that they do not interact during embryo implantation in ruminants. *Reproductive Biology and Endocrinology* **2** 19.
- Kuno N, Kadomatsu K, Fan QW, Hagihara M, Senda T, Mizutani S & Muramatsu T 1998 Female sterility in mice lacking the basigin gene, which encodes a transmembrane glycoprotein belonging to the immunoglobulin superfamily. *FEBS Letters* **425** 191–194.
- Ladner MB, Martin GA, Noble JA, Nikoloff DM, Tal R, Kawasaki ES & White TJ 1987 Human CSF-1: gene structure and alternative splicing of mRNA precursors. *Embo Journal* **6** 2693–2698.
- Laird SM, Tuckerman EM, Dalton CF, Dunphy BC, Li TC & Zhang X 1997 The production of leukaemia inhibitory factor by human endometrium: presence in uterine flushings and production by cells in culture. *Human Reproduction* **12** 569–574.
- Lee RS, Li N, Ledger AM & Pollard JW 2003 Dynamic regulation of expression of colony-stimulating factor 1 in the reproductive tract of cattle during the estrous cycle and in pregnancy. *Biology of Reproduction* **69** 518–528.
- Lessey BA 1994 The use of integrins for the assessment of uterine receptivity. *Fertility and Sterility* **61** 812–814.
- Lessey BA & Castelbaum AJ 1998 Integrins and endometriosis: fact or artefact? *Human Reproduction* **13** 3578–3580.
- Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albelda SM & Buck CA 1992 Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. *Journal of Clinical Investigation* **90** 188–195.
- Lessey BA, Castelbaum AJ, Sawin SW, Buck CA, Schinnar R, Bilker W & Strom BL 1994 Aberrant integrin expression in the endometrium of women with endometriosis. *Journal of Clinical Endocrinology and Metabolism* **79** 643–649.
- Lessey BA, Castelbaum AJ, Sawin SW & Sun J 1995 Integrins as markers of uterine receptivity in women with primary unexplained infertility. *Fertility and Sterility* **63** 535–542.

- Lessey BA, Yeh I, Castelbaum AJ, Fritz MA, Ilesanmi AO, Korzeniowski P, Sun J & Chwalisz K 1996 Endometrial P4 receptors and markers of uterine receptivity in the window of implantation. *Fertility and Sterility* **65** 477–483.
- Li S, Chiang TC, Davis GR, Williams RM, Wilson VP & McLachlan JA 2001 Decreased expression of Wnt7a mRNA is inversely associated with the expression of E2 receptor- α in human uterine leiomyoma. *Journal of Clinical Endocrinology and Metabolism* **86** 454–457.
- Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM & Dey SK 1997 Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* **91** 197–208.
- Lim H, Gupta RA, Ma WG, Paria BC, Moller DE, Morrow JD, DuBois RN, Trzaskos JM & Dey SK 1999a Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPAR δ . *Genes and Development* **13** 1561–1574.
- Lim H, Ma L, Ma WG, Maas RL & Dey SK 1999b Hoxa-10 regulates uterine stromal cell responsiveness to P4 during implantation and decidualization in the mouse. *Molecular Endocrinology* **13** 1005–1017.
- Luetke NC, Qiu TH, Fenton SE, Troyer KL, Riedel RF, Chang A & Lee DC 1999 Targeted inactivation of the EGF and amphiregulin genes reveals distinct roles for EGF receptor ligands in mouse mammary gland development. *Development* **126** 2739–2750.
- Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, Shyamala G, Conneely OM & O'Malley BW 1995 Mice lacking P4 receptor exhibit pleiotropic reproductive abnormalities. *Genes and Development* **9** 2266–2278.
- Ma L, Benson GV, Lim H, Dey SK & Maas RL 1998 Abdominal (B) AbdB Hox genes regulation in adult uterus by estrogen and progesterone and repression in müllerian duct by the synthetic estrogen diethylstilbestrol. *Developmental Biology* **197** 141–154.
- Makker A, Singh MM, Chowdhury SR, Maitra SC & Kamboj VP 1994 Uterine estradiol and P4 receptor concentration in relation to circulating hormone levels and histoarchitecture during high endometrial sensitivity and induced decidualization in guinea pigs. *Journal of Steroid Biochemistry and Molecular Biology* **48** 535–543.
- Mann GB, Fowler KJ, Gabriel A, Nice EC, Williams RL & Dunn AR 1993 Mice with a null mutation of the TGF α gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. *Cell* **73** 249–261.
- Marions L & Danielsson KG 1999 Expression of cyclo-oxygenase in human endometrium during the implantation period. *Molecular Human Reproduction* **5** 961–965.
- Matsumoto H, Ma WG, Daikoku T, Zhao X, Paria BC, Das SK, Trzaskos JM & Dey SK 2002a Cyclooxygenase-2 differentially directs uterine angiogenesis during implantation in mice. *Journal of Biological Chemistry* **277** 29260–29267.
- Matsumoto H, Zhao X, Das SK, Hogan BL & Dey SK 2002b Indian hedgehog as a P4-responsive factor mediating epithelial-mesenchymal interactions in the mouse uterus. *Developmental Biology* **245** 280–290.
- McGinnis W & Krumlauf R 1992 Homeobox genes and axial patterning. *Cell* **68** 283–302.
- McGinnis W, Levine MS, Hafen E, Kuroiwa A & Gehring WJ 1984 A conserved DNA sequence in homoeotic genes of the Drosophila Antennapedia and bithorax complexes. *Nature* **308** 428–433.
- McHugh KP, Hodivala-Dilke K, Zheng MH, Namba N, Lam J, Novack D, Feng X, Ross FP, Hynes RO & Teitelbaum SL 2000 Mice lacking beta3 integrins are osteosclerotic because of dysfunctional osteoclasts. *Journal of Clinical Investigation* **105** 433–440.
- McLaren A 1990 *The Embryo*. Cambridge, UK: Cambridge University Press.
- Mericskay M, Kitajewski J & Sassoon D 2004 Wnt5a is required for proper epithelial-mesenchymal interactions in the uterus. *Development* **131** 2061–2072.
- Meseguer M, Aplin JD, Caballero-Campo P, O'Connor JE, Martin JC, Remohi J, Pellicer A & Simon C 2001 Human endometrial mucin MUC1 is up-regulated by P4 and down-regulated *in vitro* by the human blastocyst. *Biology of Reproduction* **64** 590–601.
- Meyer WR, Castelbaum AJ, Somkuti S, Sagoskin AW, Doyle M, Harris JE & Lessey BA 1997 Hydrosalpinges adversely affect markers of endometrial receptivity. *Human Reproduction* **12** 1393–1398.
- Miller C & Sassoon DA 1998 Wnt-7a maintains appropriate uterine patterning during the development of the mouse female reproductive tract. *Development* **125** 3201–3211.
- Miller C, Pavlova A & Sassoon DA 1998 Differential expression patterns of Wnt genes in the murine female reproductive tract during development and the estrous cycle. *Mechanisms of Development* **76** 91–99.
- Mizukami IF, Vinjamuri SD, Perini F, Liu DY & Todd RF III 1991 Purification, biochemical composition, and biosynthesis of the Mo3 activation antigen expressed on the plasma membrane of human mononuclear phagocytes. *Journal of Immunology* **147** 1331–1337.
- Modric T, Kowalski AA, Green ML, Simmen RC & Simmen FA 2000 Pregnancy-dependent expression of leukaemia inhibitory factor (LIF), LIF receptor-beta and interleukin-6 (IL-6) messenger ribonucleic acids in the porcine female reproductive tract. *Placenta* **21** 345–353.
- Moudgal NR & Ravindranath N 1989 Requirement for E2 in implantation and post implantation survival of blastocyst in the bonnet monkey. *Progress in Clinical and Biological Research* **294** 277–288.
- Mueller MD, Vigne JL, Minchenko A, Lebovic DI, Leitman DC & Taylor RN 2000 Regulation of vascular endothelial growth factor (VEGF) gene transcription by E2 receptors alpha and beta. *PNAS* **97** 10972–10977.
- Mulac-Jericevic B, Mullinax RA, DeMayo FJ, Lydon JP & Conneely OM 2000 Subgroup of reproductive functions of P4 mediated by P4 receptor-B isoform. *Science* **289** 1751–1754.
- Mulac-Jericevic B, Lydon JP, DeMayo FJ & Conneely OM 2003 Defective mammary gland morphogenesis in mice lacking the P4 receptor B isoform. *PNAS* **100** 9744–9749.
- Olson GE, Winfrey VP, Matrisian PE, Melner MH & Hoffman LH 1997 Specific expression of haptoglobin mRNA in implantation-stage rabbit uterine epithelium. *Journal of Endocrinology* **152** 69–80.
- Orderer C, Cypriani B, Vuillermoz C & Adessi GL 1993 Epidermal growth factor and insulin induce the proliferation of guinea pig endometrial stromal cells in serum-free culture, whereas estradiol and P4 do not. *Biology of Reproduction* **49** 1032–1044.
- Oshima K, Watanabe H, Yoshihara K, Kojima T, Dochi O, Takenouchi N, Fukushima M & Komatsu M 2003 Gene expression of leukemia inhibitory factor (LIF) and macrophage colony stimulating factor (M-CSF) in bovine endometrium during early pregnancy. *Theriogenology* **60** 1217–1226.
- Ota H, Igarashi S, Sasaki M & Tanaka T 2001 Distribution of cyclooxygenase-2 in eutopic and ectopic endometrium in endometriosis and adenomyosis. *Human Reproduction* **16** 561–566.
- Paria BC, Das SK, Andrews GK & Dey SK 1993 Expression of the epidermal growth factor receptor gene is regulated in mouse blastocysts during delayed implantation. *PNAS* **90** 55–59.
- Paria BC, Das SK, Huet-Hudson YM & Dey SK 1994 Distribution of transforming growth factor alpha precursors in the mouse uterus during the periimplantation period and after steroid hormone treatments. *Biology of Reproduction* **50** 481–491.
- Paria BC, Elenius K, Klagsbrun M & Dey SK 1999 Heparin-binding EGF-like growth factor interacts with mouse blastocysts independently of ErbB1: a possible role for heparan sulfate proteoglycans and ErbB4 in blastocyst implantation. *Development* **126** 1997–2005.
- Paria BC, Ma W, Tan J, Raja S, Das SK, Dey SK & Hogan BL 2001 Cellular and molecular responses of the uterus to embryo implantation can be elicited by locally applied growth factors. *PNAS* **98** 1047–1052.

- Pfeiffer D, Spranger J, Al-Deiri M, Kimmig R, Fisseler-Eckhoff A, Scheidel P, Schatz H, Jensen A & Pfeiffer A 1997 mRNA expression of ligands of the epidermal-growth-factor-receptor in the uterus. *International Journal of Cancer* **72** 581–586.
- Pollard JW 1997 Role of colony-stimulating factor-1 in reproduction and development. *Molecular Reproduction and Development* **46** 54–60; discussion 60–61.
- Pollard JW, Bartocci A, Arceci R, Orlofsky A, Ladner MB & Stanley ER 1987 Apparent role of the macrophage growth factor, CSF-1, in placental development. *Nature* **330** 484–486.
- Pollard JW, Hunt JS, Wiktor-Jedrzejczak W & Stanley ER 1991 A pregnancy defect in the osteopetrotic (op/op) mouse demonstrates the requirement for CSF-1 in female fertility. *Developmental Biology* **148** 273–283.
- Reese J, Brown N, Das SK & Dey SK 1998 Expression of new differentiation factor during the periimplantation period in the mouse uterus. *Biology of Reproduction* **58** 719–727.
- Reynolds LP, Kirsch JD, Kraft KC & Redmer DA 1998 Time-course of the uterine response to estradiol-17 β in ovariectomized ewes: expression of angiogenic factors. *Biology of Reproduction* **59** 613–620.
- Robb L, Li R, Hartley L, Nandurkar HH, Koentgen F & Begley CG 1998 Infertility in female mice lacking the receptor for interleukin 11 is due to a defective uterine response to implantation. *Nature Medicine* **4** 303–308.
- Rowe AJ, Wulff C & Fraser HM 2003 Localization of mRNA for vascular endothelial growth factor (VEGF), angiopoietins and their receptors during the peri-implantation period and early pregnancy in marmosets (*Callithrix jacchus*). *Reproduction* **126** 227–238.
- Sands BE, Bank S, Sninsky CA, Robinson M, Katz S, Singleton JW, Miner PB, Safdi MA, Galanduk S, Hanauer SB, Varilek GW, Buchman AL, Rodgers VD, Salzberg B, Cai B, Loewy J, DeBruin MF, Rogge H, Shapiro M & Schwertschlag US 1999 Preliminary evaluation of safety and activity of recombinant human interleukin 11 in patients with active Crohn's disease. *Gastroenterology* **117** 58–64.
- Satokata I, Benson G & Maas R 1995 Sexually dimorphic sterility phenotypes in Hoxa10-deficient mice. *Nature* **374** 460–463.
- Savage CR Jr, Hash JH & Cohen S 1973 Epidermal growth factor. Location of disulfide bonds. *Journal of Biological Chemistry* **248** 7669–7672.
- Schultz JF, Mayernik L, Rout UK & Armant DR 1997 Integrin trafficking regulates adhesion to fibronectin during differentiation of mouse peri-implantation blastocysts. *Developmental Genetics* **21** 31–43.
- Schuster VL, Lu R, Kanai N, Bao Y, Rosenberg S, Prie D, Ronco P & Jennings ML 1996 Cloning of the rabbit homologue of mouse 'basigin' and rat 'OX-47': kidney cell type-specific expression, and regulation in collecting duct cells. *Biochimica et Biophysica Acta* **1311** 13–19.
- Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML & Schuh AC 1995 Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* **376** 62–66.
- Shifren JL, Tseng JF, Zaloudek CJ, Ryan IP, Meng YG, Ferrara N, Jaffe RB & Taylor RN 1996 Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis. *Journal of Clinical Endocrinology and Metabolism* **81** 3112–3118.
- Shinetugs B, Runesson E, Bonello NP, Brannstrom M & Norman RJ 1999 Colony stimulating factor-1 concentrations in blood and follicular fluid during the human menstrual cycle and ovarian stimulation: possible role in the ovulatory process. *Human Reproduction* **14** 1302–1306.
- Simon C, Valbuena D, Krussel J, Bernal A, Murphy CR, Shaw T, Pelliger A & Polan ML 1998 Interleukin-1 receptor antagonist prevents embryonic implantation by a direct effect on the endometrial epithelium. *Fertility and Sterility* **70** 896–906.
- Smith CA, Moore HD & Hearn JP 1987 The ultrastructure of early implantation in the marmoset monkey (*Callithrix jacchus*). *Anatomy and Embryology (Berl)* **175** 399–410.
- Smith WL & Dewitt DL 1996 Prostaglandin endoperoxide H synthases-1 and -2. *Advances in Immunology* **62** 167–215.
- Song H, Lim H, Das SK, Paria BC & Dey SK 2000 Dysregulation of EGF family of growth factors and COX-2 in the uterus during the preattachment and attachment reactions of the blastocyst with the luminal epithelium correlates with implantation failure in LIF-deficient mice. *Molecular Endocrinology* **14** 1147–1161.
- Spencer TE, Stagg AG, Joyce MM, Jenster G, Wood CG, Bazer FW, Wiley AA & Bartol FF 1999 Discovery and characterization of endometrial epithelial messenger ribonucleic acids using the ovine uterine gland knockout model. *Endocrinology* **140** 4070–4080.
- Stanley ER, Guilbert LJ, Tushinski RJ & Bartelmez SH 1983 CSF-1 – a mononuclear phagocyte lineage-specific hemopoietic growth factor. *Journal of Cellular Biochemistry* **21** 151–159.
- Steck T, Giess R, Suetterlin MW, Bolland M, Wiest S, Poehls UG & Dietl J 2004 Leukaemia inhibitory factor (LIF) gene mutations in women with unexplained infertility and recurrent failure of implantation after IVF and embryo transfer. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **112** 69–73.
- Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Kontgen F & Abbondanzo SJ 1992 Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature* **359** 76–79.
- St-Jacques B, Hammerschmidt M & McMahon AP 1999 Indian hedgehog signaling regulates proliferation and differentiation of chondrocytes and is essential for bone formation. *Genes and Development* **13** 2072–2086.
- Strakova Z, Srisuparp S & Fazleabas AT 2002 IL-1 β during *in vitro* decidualization in primate. *Journal of Reproductive Immunology* **55** 35–47.
- Sun T, Li SJ, Diao HL, Teng CB, Wang HB & Yang ZM 2004 Cyclooxygenases and prostaglandin E synthases in the endometrium of the rhesus monkey during the menstrual cycle. *Reproduction* **127** 465–473.
- Surveyor GA, Gendler SJ, Pemberton L, Das SK, Chakraborty I, Julian J, Pimental RA, Wegner CC, Dey SK & Carson DD 1995 Expression and steroid hormonal control of Muc-1 in the mouse uterus. *Endocrinology* **136** 3639–3647.
- Sutherland AE, Calarco PG & Damsky CH 1993 Developmental regulation of integrin expression at the time of implantation in the mouse embryo. *Development* **119** 1175–1186.
- Takamoto N, Zhao B, Tsai SY & DeMayo FJ 2002 Identification of Indian hedgehog as a P4-responsive gene in the murine uterus. *Molecular Endocrinology* **16** 2338–2348.
- Tamada H, Tsubutani D, Kawate N, Inaba T, Matsuyama S, Imakawa K, Sakai S, Christenson RK & Sawada T 2002 Detection of transforming growth factor- α and epidermal growth factor receptor mRNA and immunohistochemical localization of their proteins in the ovine uterus during the early implantation period. *Histochemical Journal* **34** 383–390.
- Tarantal AF & Hendrickx AG 1988 Use of ultrasound for early pregnancy detection in the rhesus and cynomolgus macaque (*Macaca mulatta* and *Macaca fascicularis*). *Journal of Medical Primatology* **17** 105–112.
- Tarantal AF, Laughlin LS, Dieter J, Tieu J, Hendrickx AG, Overstreet JW & Lasley BL 1997 Pregnancy detection by ultrasound and chorionic gonadotropin during the peri-implantation period in the macaque (*Macaca fascicularis*). *Early Pregnancy* **3** 281–290.
- Taylor HS, Vanden Heuvel GB & Igarashi P 1997 A conserved Hox axis in the mouse and human female reproductive system: late establishment and persistent adult expression of the Hoxa cluster genes. *Biology of Reproduction* **57** 1338–1345.
- Taylor HS, Arici A, Olive D & Igarashi P 1998 HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. *Journal of Clinical Investigation* **101** 1379–1384.

- Taylor HS, Bagot C, Kardana A, Olive D & Arici A 1999 HOX gene expression is altered in the endometrium of women with endometriosis. *Human Reproduction* **14** 1328–1331.
- Thapar M, Kumari GL, Shrivastav TG & Pandey PK 1988 Hormonal control of implantation in guinea pigs. *Steroids* **52** 85–108.
- Timmreck LS, Pan HA, Reindollar RH & Gray MR 2003 WNT7A mutations in patients with Mullerian duct abnormalities. *Journal of pediatric and adolescent gynecology* **16** 217–221.
- Tischer E, Mitchell R, Hartman T, Silva M, Gospodarowicz D, Fiddes JC & Abraham JA 1991 The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. *Journal of Biological Chemistry* **266** 11947–11954.
- Torrey DS, Holt VJ, Keenan JA, Harris G, Caudle MR & Torrey RJ 1996 Vascular endothelial growth factor expression in cycling human endometrium. *Fertility and Sterility* **66** 72–80.
- Troyer KL, Luetkeke NC, Saxon ML, Qiu TH, Xian CJ & Lee DC 2001 Growth retardation, duodenal lesions, and aberrant ileum architecture in triple null mice lacking EGF, amphiregulin, and TGF- α . *Gastroenterology* **121** 68–78.
- Tsark EC, Adamson ED, Withers GE III & Wiley LM 1997 Expression and function of amphiregulin during murine preimplantation development. *Molecular Reproduction and Development* **47** 271–283.
- Tulac S, Nayak NR, Kao LC, Van Waes M, Huang J, Lobo S, Germeyer A, Lessey BA, Taylor RN, Suchanek E & Giudice LC 2003 Identification, characterization, and regulation of the canonical Wnt signaling pathway in human endometrium. *Journal of Clinical Endocrinology and Metabolism* **88** 3860–3866.
- Tuo W, Harney JP & Bazer FW 1995 Colony-stimulating factor-1 in conceptus and uterine tissues in pigs. *Biology of Reproduction* **53** 133–142.
- Ullrich A & Schlessinger J 1990 Signal transduction by receptors with tyrosine kinase activity. *Cell* **61** 203–212.
- Uotila PJ, Erkkola RU & Klemi PJ 2002 The expression of cyclooxygenase-1 and -2 in proliferative endometrium and endometrial adenocarcinoma. *Annals of Medicine* **34** 428–433.
- Vainio S, Heikkilä M, Kispert A, Chin N & McMahon AP 1999 Female development in mammals is regulated by Wnt-4 signalling. *Nature* **397** 405–409.
- Vogiagis D, Fry RC, Sandeman RM & Salamonsen LA 1997 Leukaemia inhibitory factor in endometrium during the oestrous cycle, early pregnancy and in ovariectomized steroid-treated ewes. *Journal of Reproduction and Fertility* **109** 279–288.
- Wang W, Van de Water T & Lufkin T 1998 Inner ear and maternal reproductive defects in mice lacking the Hmx3 homeobox gene. *Development* **125** 621–634.
- Wang H, Li Q, Lin H, Yu X, Qian D, Dai J, Duan E & Zhu C 2003 Expression of vascular endothelial growth factor and its receptors in the rhesus monkey (*Macaca mulatta*) endometrium and placenta during early pregnancy. *Molecular Reproduction and Development* **65** 123–131.
- Wang H, Ma WG, Tejada L, Zhang H, Morrow JD, Das SK & Dey SK 2004 Rescue of female infertility from the loss of cyclooxygenase-2 by compensatory up-regulation of cyclooxygenase-1 is a function of genetic makeup. *Journal of Biological Chemistry* **279** 10649–10658.
- Welter H, Wollenhaupt K, Tiemann U & Einspanier R 2003 Regulation of the VEGF-system in the endometrium during steroid-replacement and early pregnancy of pigs. *Experimental and Clinical Endocrinology and Diabetes* **111** 33–40.
- Wesseling J, Van der Valk SW, Vos HL, Sonnenberg A & Hilken J 1995 Episialin (MUC1) overexpression inhibits integrin-mediated cell adhesion to extracellular matrix components. *Journal of Cellular Biology* **129** 255–265.
- Wetzka B, Nusing R, Charnock-Jones DS, Schafer W, Zahradnik HP & Smith SK 1997 Cyclooxygenase-1 and -2 in human placenta and placental bed after normal and pre-eclamptic pregnancies. *Human Reproduction* **12** 2313–2320.
- Wiktor-Jedrzejczak W, Urbanowska E, Aukerman SL, Pollard JW, Stanley ER, Ralph P, Ansari AA, Sell KW & Szperl M 1991 Correction by CSF-1 of defects in the osteopetrotic (op/op) mouse suggests local, developmental, and humoral requirements for this growth factor. *Experimental Hematology* **19** 1049–1054.
- Wilcox AJ, Baird DD & Weinberg CR 1999 Time of implantation of the conceptus and loss of pregnancy. *New England Journal of Medicine* **340** 1796–1799.
- Wiley AA, Bartol FF & Barron DH 1987 Histogenesis of the ovine uterus. *Journal of Animal Science* **64** 1262–1269.
- Wimsatt WA 1975 Some comparative aspects of implantation. *Biology of Reproduction* **12** 1–40.
- Wodarz A & Nusse R 1998 Mechanisms of Wnt signaling in development. *Annual Review of Cell Developmental Biology* **14** 59–88.
- Wooding FB 1984 Role of binucleate cells in fetomaternal cell fusion at implantation in the sheep. *American Journal of Anatomy* **170** 233–250.
- Wooding FB 1992 Current topic: the synepitheliochorial placenta of ruminants: binucleate cell fusions and hormone production. *Placenta* **13** 101–113.
- Xiao LJ, Chang H, Ding NZ, Ni H, Kadomatsu K & Yang ZM 2002a Basigin expression and hormonal regulation in mouse uterus during the peri-implantation period. *Molecular Reproduction and Development* **63** 47–54.
- Xiao LJ, Diao HL, Ma XH, Ding NZ, Kadomatsu K, Muramatsu T & Yang ZM 2002b Basigin expression and hormonal regulation in the rat uterus during the peri-implantation period. *Reproduction* **124** 219–225.
- Xu P, Wang Y, Piao Y, Bai S, Xiao Z, Jia Y, Luo S & Zhuang L 2001 Effects of matrix proteins on the expression of matrix metalloproteinase-2, -9, and -14 and tissue inhibitors of metalloproteinases in human cytotrophoblast cells during the first trimester. *Biology of Reproduction* **65** 240–246.
- Yamaguchi TP 2001 Heads or tails: Wnts and anterior-posterior patterning. *Current Biology* **11** R713–R724.
- Yamaguchi TP, Bradley A, McMahon AP & Jones S 1999 A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* **126** 1211–1223.
- Yang ZM, Le SP, Chen DB, Yasukawa K & Harper MJ 1995 Expression patterns of leukaemia inhibitory factor receptor (LIFR) and the gp130 receptor component in rabbit uterus during early pregnancy. *Journal of Reproduction and Fertility* **103** 249–255.
- Yarden Y 2001 The EGFR family and its ligands in human cancer. Signalling mechanisms and therapeutic opportunities. *European Journal of Cancer* **37** (Suppl 4) S3–S8.
- Yoo HJ, Barlow DH & Mardon HJ 1997 Temporal and spatial regulation of expression of heparin-binding epidermal growth factor-like growth factor in the human endometrium: a possible role in blastocyst implantation. *Developmental Genetics* **21** 102–108.
- Yue ZP, Yang ZM, Li SJ, Wang HB & Harper MJ 2000 Epidermal growth factor family in rhesus monkey uterus during the menstrual cycle and early pregnancy. *Molecular Reproduction and Development* **55** 164–174.
- Zhang H & Bradley A 1996 Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. *Development* **122** 2977–2986.
- Zhou Y, McMaster M, Woo K, Janatpour M, Perry J, Karpanen T, Alitalo K, Damsky C & Fisher SJ 2002 Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. *American Journal of Pathology* **160** 1405–1423.
- Zhu LJ, Bagchi MK & Bagchi IC 1998 Attenuation of calcitonin gene expression in pregnant rat uterus leads to a block in embryonic implantation. *Endocrinology* **139** 330–339.