Roadmap to embryo implantation: clues from mouse models

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Abstract | Implantation involves an intricate discourse between the embryo and uterus and is a gateway to further embryonic development. Synchronizing embryonic development until the blastocyst stage with the uterine differentiation that takes place to produce the receptive state is crucial to successful implantation, and therefore to pregnancy outcome. Although implantation involves the interplay of numerous signalling molecules, the hierarchical instructions that coordinate the embryo–uterine dialogue are not well understood. This review highlights our knowledge about the molecular development of preimplantation and implantation and the future challenges of the field. A better understanding of periimplantation biology could alleviate female infertility and help to develop novel contraceptives.

Blastocyst
An embryonic stage in mammals that is derived from a morula and is comprised of a fluid-filled cavity (blastocoel) and two cell types, the inner cell mass and the trophectoderm.

The implantation of the blastocyst into the maternal uterus is a crucial step in mammalian reproduction and, like many developmental processes, it involves an intricate succession of genetic and cellular interactions, all of which must be executed within an optimal time frame. In mammals, the beginning of new life is seeded at fertilization. The fertilized egg undergoes many cell divisions to form a blastocyst (BOX 1, part a). These developmental events are synchronized with the proliferation and differentiation of specific uterine cell types, primarily under the direction of ovarian oestrogen and progesterone (P_4). These hormones make the uterus conducive ('receptive') to accept a blastocyst for implantation[1–4] (BOX 2).

A reciprocal interaction between the blastocyst and receptive uterus is essential for implantation. Early pregnancy loss in humans, which often occurs due to defects that occur before, during or immediately after implantation, is a worldwide social and economic concern. Although the human population is growing rapidly and will probably reach nine billion by 2050, 15% of couples worldwide are childless because of infertility. Many underlying causes of human infertility have been overcome by *in vitro* fertilization and embryo-transfer techniques; implantation rates, however, remain disappointingly low, probably owing to embryos being transferred into a nonreceptive uterus. There is, therefore, a continuing need to unravel the complexities of preimplantation embryonic development and implantation to address two contrasting global issues: to improve infertility, and to develop novel contraceptives.

Here, we focus on the molecular and genetic mechanisms of implantation that have been gleaned primarily from mouse models, and which could be relevant to humans. We describe the signalling networks that direct preimplantation embryonic development, confer blastocyst competency and uterine receptivity to implantation, instigate the blastocyst–uterine dialogue at various phases of implantation, and finally, participate in orienting the embryo–uterine axis during the postimplantation period. The clinical implications of these findings are also illustrated.

Revisiting this field is timely because of the emergence of technological advances that allow us, for example, to profile global gene and protein expression in the embryo and uterus, and to predict how molecules interact during implantation. Many gene-knockout mouse models have also provided a wealth of information that needs to be carefully interpreted in addressing human fertility.

Although the cellular events that define the various stages of implantation have been described[1,5], the molecular genetic pathways that are crucial to this process, and how they interact, are not clearly understood. Because this complex process varies across species (BOX 1, part b)[1], the formulation of a unified model for the molecular basis of implantation in mammals seems unrealistic at present. However, inroads can be made by addressing a few crucial questions to determine: which signalling pathways are crucial; which are complementary or antagonistic; and how these pathways are coordinated. Another challenge is to identify the signalling pathways that have a limited role during normal pregnancy, but that become important

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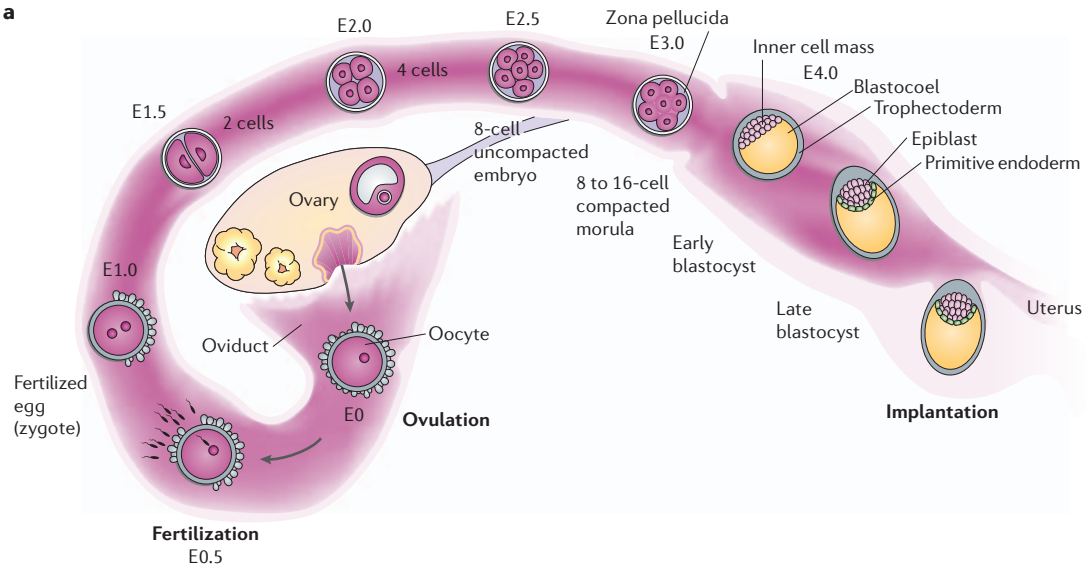
under conditions of stress. Answers to these questions might help to improve fertility and fertility-associated issues in women.

Preimplantation embryonic development

The development of the mammalian preimplantation embryo encompasses the period from fertilization to implantation. This period is marked by three principal transitions, all of which involve dynamic genetic programming: fertilization and the first cell division; continued cell division; the establishment of cell polarity and compaction to form a morula; and lineage differentiation to form a blastocyst (followed by implantation).

At the blastocyst stage, embryos mature and escape from their outer shells (zona pellucidae) and then gain implantation competency. The mature blastocyst is composed of three cell types: the outer epithelial trophectoderm (Tr), the primitive endoderm (PE) and the pluripotent inner cell mass (ICM) (BOX 1, part a). The ICM generates future cell lineages of the embryo proper,

Box 1 | **Preimplantation and implantation events**



a | Preimplantation embryo development and implantation in mice. Following fertilization in the oviduct, the embryo undergoes several rounds of mitotic cell division, ultimately forming a ball of cells called a morula. At the late morula stage, the embryo enters the uterine lumen and transforms into a blastocyst that contains a cavity (called blastocoel) with two distinct cell populations, the inner cell mass (ICM) and the trophectoderm (the progenitor of trophoblast cells). Before implantation, the blastocyst escapes from its outer shell (the zona pellucida) and differentiates to produce additional cell types — the epiblast and the primitive endoderm. At this stage, the trophectoderm attaches to the uterine lining to initiate the process of implantation. E, embryonic day. **b** | Implantation strategies in different species. The main purpose of implantation is to ensure that trophoblast cells firmly anchor into the endometrial stroma. Ultrastructural studies have revealed that there are different modes of implantation in mammals : the trophectoderm-derived trophoblast (T) cells can breach the uterine luminal epithelium, coalesce with it or trespass between the uterine cells to home in on the underlying stroma. In mice and rats, the attachment of the blastocyst (represented by En, the embryonic endoderm) to the luminal epithelium (LE) imparts epithelial apoptosis locally at the site of attachment, facilitating the penetration of trophoblast cells through the LE layer into the stroma (S). In guinea pigs, the syncytial trophoblast makes focal protrusions through the zona pellucida (ZP) and intrudes between epithelial cells, ultimately embedding the embryo in the uterine stroma. In rabbits, clusters of trophoblast cells (trophoblastic knobs) fuse with the LE (T–LE fusion) to form symplasma. In primates, the syncytial trophoblast is formed near the ICM, which intrudes between uterine epithelial cells and penetrates the basal lamina. D, decidual cells. Part **a** is adapted with permission from REF. 88 © (2001) Terese Winslow. Part **b** is adapted with permission from REF. 1 © (2000) Elsevier Science.

Compaction
An embryonic state in which the cells of the morula are flattened and cell outlines are not clearly distinguishable.

Morula
A cluster of blastomeres that results from the early cleavages of a zygote.

Zona pellucida
An outer shell composed of glycoproteins that encircles oocytes or preimplantation embryos.

Trophectoderm
The outer layer of the blastocyst that is the progenitor of future trophoblast cell types.

Inner cell mass
Cells that are present inside the blastocyst. These cells are pluripotent and give rise to the embryo proper (that is, the cells that are not destined to become the placenta).

Syncytial trophoblast
The syncytial multinucleated outer layer of the trophoblast.

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mRNA differential display
A technique for detecting genes that are expressed only under specific conditions; it involves isolating mRNA from two or more cell populations and comparing their transcript-expression levels.

Pseudopregnancy
A condition similar to pregnancy, without the presence of a fertilized egg, which is produced by sterile mating or hormone treatment.

while the Tr makes the first physical and physiological connection with the luminal epithelium (LE) of the maternal uterus for implantation.

Fertilization and first cell division: maternal to zygotic expression. A unique feature of preimplantation embryonic development is the presence of maternally stored RNAs and proteins in mature, unfertilized eggs. In mice, fertilization triggers the degradation of oocyte-stored transcripts, which is 90% complete by the 2-cell stage[6]. Transcription from the newly formed zygotic genome, known as zygotic genome activation (ZGA), establishes the gene-expression patterns that are required for continued development. A comprehensive molecular characterization of the developmental reprogramming from maternal to zygotic gene expression has been hindered by the scarcity of embryonic tissues and lack of appropriate molecular approaches. Conventional methods, such as reverse-trancriptase PCR, western blotting and immunohistochemistry have been used to examine the expression patterns of a limited number of genes, but not the dynamic changes that occur during early development. To assess more robust gene-expression patterns, high-resolution 2D protein gels[7,8], mRNA differential display[9] and the analysis of expressed sequence tags (ESTs) that were derived from libraries of several preimplantation stages[10] were carried out. However, these studies provided information on signature transcripts and/or proteins, but not global gene expression or proteome profiles.

Recently, global gene-expression profiles that were derived from microarray experiments have generated a comprehensive data set that covers nearly all mouse genes during preimplantation development[11–13]. One remarkable finding is the existence of programmed waves of upregulated and downregulated gene expression, which parallels the stages of embryonic development. According to Hamatani *et al.*[11], maternal-to-zygotic gene activation shows two principal transient waves of *de novo* transcription. The first wave corresponds to the 'major ZGA', which peaks between the 2- and 4-cell stages and leads to the most marked genetic reprogramming. The second wave, mid-preimplantation gene activation (MGA), peaks at the 8-cell stage and precedes the morula-to-blastocyst formation (FIG. 1a)[11]; indeed, MGA involves the expression of intercellular adhesion molecules during blastomere polarity and compaction. Irrespective of the underlying mechanisms, the identification of zygotic transcription cascades that occur at each successive phase (minor ZGA>major ZGA>MGA; FIG. 1a) is the first step towards analysing the complex gene regulatory network that governs embryonic development.

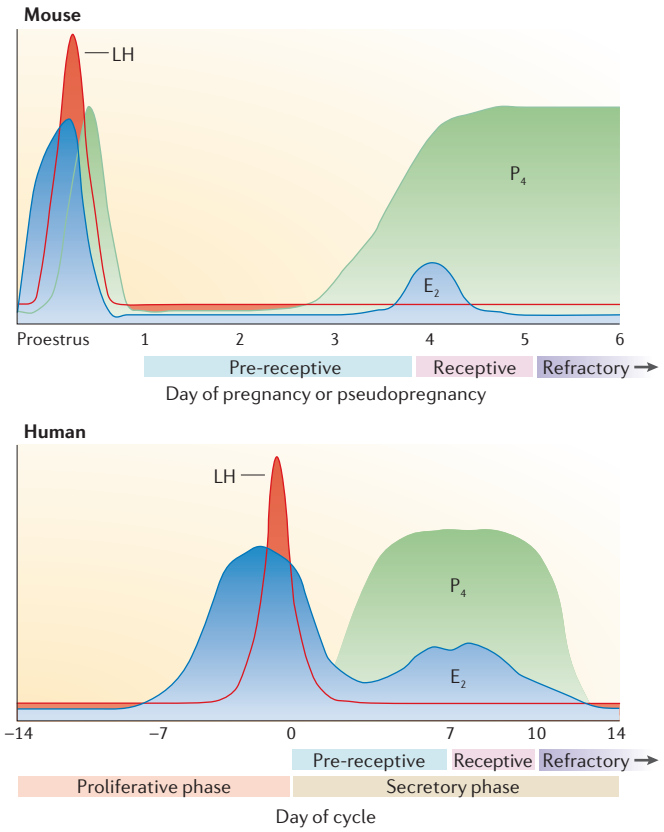
Studies of transgenic mice show that many genes have vital functions in preimplantation embryonic development, and that their functions, as inferred by gene targeting, are consistent with their gene-expression profiles (see Supplementary information S1 (table); FIG. 1a). For example, the maternal-effect gene *Mater* is detected

Box 2 | The window of uterine receptivity in mice and humans

In placental mammals, the uterus differentiates into an altered state when implantation-competent blastocysts are ready to initiate implantation. This state is called uterine receptivity for implantation and lasts for a limited time. At this stage, the uterine environment is conducive to blastocyst growth, attachment and the subsequent events of implantation. The major hormones that specify uterine receptivity are the ovarian steroids progesterone (P_4) and oestrogen (E_2). In mice, the oestrous cycle is short (~4 days) and often irregular. Therefore, it is difficult to determine the receptive phase during the cycle. Blastocyst transfers in pseudopregnant recipients were used to determine various phases of uterine sensitivity to implantation. In contrast, the menstrual cycle in women is long and the hormonal changes are more predictable, which allows the state of uterine receptivity to be determined. A surge of leutinizing hormone (LH), which is secreted from the pituitary, is essential to ovulation and in programming the secretion of oestrogen and progesterone by the ovary.

Uterine sensitivity to implantation is classified into prereceptive, receptive and nonreceptive (refractory) phases. During the prereceptive phase, the uterus is unable to initiate implantation, but the uterine environment is less hostile to blastocyst survival. In contrast, during the refractory phase, the uterine environment is unfavourable to blastocyst survival. In mice (top diagram), the uterus is receptive on day 4 of pregnancy or pseudopregnancy, whereas it is prereceptive on days 1–3, and by the afternoon of day 5 it becomes nonreceptive (refractory) to implantation.

In humans (bottom diagram), the uterus is classified histologically and functionally into proliferative (follicular) and secretory (luteal) phases during the average 28–30-day menstrual cycle. During the secretory phase, the uterus is considered prereceptive for the first ~7 days following ovulation (day 0). The uterus then becomes receptive during the mid-secretory phase, which spans 7–10 days after ovulation; the nonreceptive (refractory) phase comprises the rest of the secretory phase.



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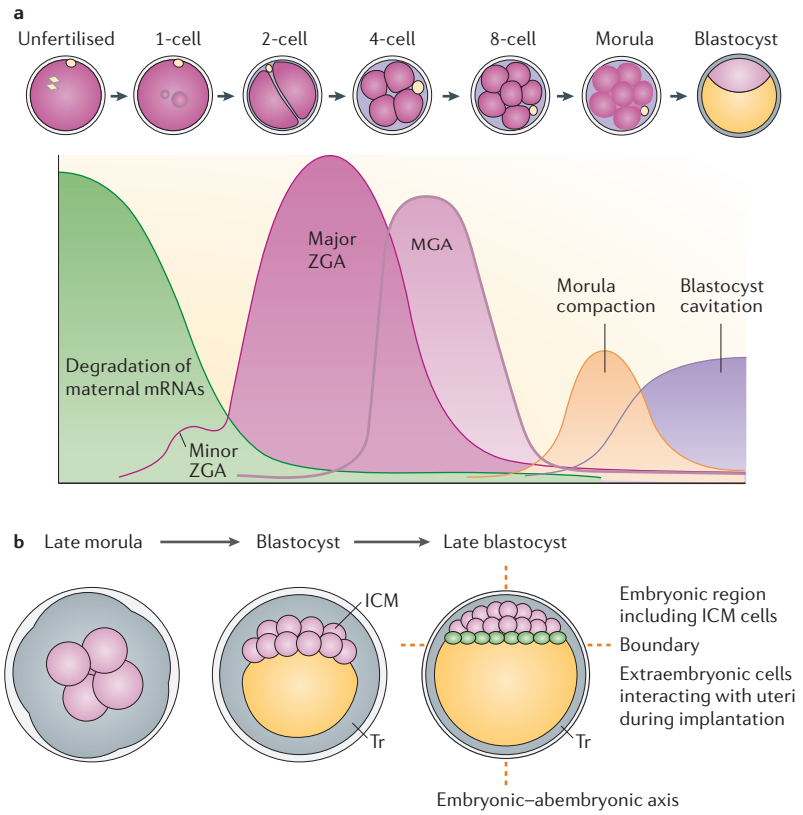

Figure 1 | **Genes governing the development of the preimplantation mouse embryo. a** | Gene expression during preimplantation embryo development. The diagram depicts the waves of gene expression that occur in preimplantation embryos, based on microarray studies, from the degradation of maternal genes, to the three successive waves of overlapping zygotic gene expression (minor and major ZGA (zygotic genome activation), and MGA (mid-preimplantation gene activation)) — following the MGA, genes that are specific to morula compaction and blastocyst cavitation are also expressed in a wave-like fashion. In addition, many genes that are expressed in embryos and stem cells are crucial to preimplantation development (see Supplementary information S1 (table)). **b,c** | Genes that are crucial for cell-fate and lineage segregations during the morula-to-blastocyst transition, as determined by expression and mutation studies in mice. Panel (**b**) represents the gene-expression patterns during blastocyst formation. Whereas *Oct4* (dark pink) is expressed throughout the embryo before the late morula stage, the expression of *Nanog* (light pink) is specifically induced in the inside cells of late morulae. *Cdx2* (blue) is expressed in the outer layer of cells in late morulae and is required for the repression of *Oct4* and *Nanog* in the trophectoderm (Tr) of the blastocyst. *Oct4* is crucial for inner-cell-mass (ICM) formation. *Gata6* (green) is expressed in the primitive endoderm of the late blastocyst, where *Oct4* and *Nanog* are repressed. Panel (**c**) shows the genetic model of lineage decision. *Oct4* represses *Cdx2* expression, which in turn represses *Oct4* expression to allow segregation of the ICM and Tr lineages of the blastocyst. An antagonism between *Nanog* and *Gata6* segregates epiblast and primitive endoderm within the ICM. Panel (**a**) is adapted with permission from REF. 11 © (2004) Elsevier Science. Panel (**b**) is adapted with permission from REF. 113 © (2005) Blackwell Publishing Ltd.

only in mature, unfertilized eggs, and its deletion restricts development beyond the 2-cell stage[14]. Other studies have identified additional preimplantation maternal and zygotic genes (see Supplementary information S1 (table); FIG. 1a). Collectively, these studies have advanced our knowledge of preimplantation embryonic development, but a comprehensive understanding of embryonic development, especially in humans, is far from complete.

Cell polarity. Preimplantation development involves the transition of totipotent, fertilized eggs to blastocysts that contain both pluripotent ICM cells and the Tr, which is the progenitor of the trophoblast. One fundamental question, discussed in this section, is how the cellular polarity of the embryonic–abembryonic (Em–Ab) axis is established with the formation of a blastocyst (FIG. 1b). The traditional opinion is that embryonic development is symmetrical before implantation because each blastomere in 8- or 16-cell mouse embryos can produce an offspring. However, recent studies have revealed asymmetries in the potential of cells, even at very early stages (BOX 3).

Lineage differentiation. Irrespective of the debate about polarity, the molecular crosstalk that segregates and differentiates the ICM and Tr lineages in blastocysts is essential to implantation because it is the Tr that initiates this process together with the LE. Microarray and deletion studies have identified several genes that are crucial for these two cell-lineage segregations (see Supplementary information S1 (table)), which include those that encode many transcription factors, such as OCT4, SOX2, NANOG, CDX2 and Eomesodermin (EOMES)[15,16] (FIG. 1b,c).

ICM formation depends on OCT4, which is encoded by *Pou5f1*, as *Pou5f1*-mutant blastocysts contain only Tr. *Pou5f1* is restricted to the ICM at the blastocyst stage[17]. SOX2, a high-mobility group (HMG)-box transcription factor, shows a similar expression profile to *Pou5f1*, and together they prevent trophoblast specification[18]. However, the inability of OCT4 alone to maintain embryonic-stem-cell (ES cell) pluripotency in the absence of leukaemia inhibitory factor (LIF)[19] indicates that other pluripotency-promoting factors, such as NANOG (a homeobox (Hox) transcription factor), are involved. The consensus is that, while both NANOG and OCT4 are required for ICM specification, they suppress the formation of extraembryonic lineages; OCT4 represses trophoblast and NANOG parietal–visceral endoderm formation[20,21]. A recent genome-scale location analysis in human ES cells has revealed a novel mechanism for establishing pluripotency. It showed that OCT4, SOX2 and NANOG co-occupy a substantial portion of their target genes and collaborate to form a circuitry of autoregulatory and feed-forward loops[22].

It has been proposed that the Tr develops by default in the absence of OCT4. However, CDX2, a caudal-type homeodomain protein, is crucial for segregating ICM and Tr lineages at the blastocyst stage by ensuring the

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Box 3 | Cell polarity in the early embryo: an ongoing debate

Specific labelling of blastomeres or zona pellucidae indicates that the plane of the first cleavage specifies embryonic polarity. That is, the plane of cleavage is orthogonal to the future embryonic–abembryonic axis of the blastocyst, with one blastomere predominantly contributing to the embryonic pole (polar trophectoderm (Tr) and deeper inner-cell-mass (ICM) cells) and the other to the abembryonic pole (mural Tr and more superficial ICM)[89–93]. This indicates a developmental asymmetry at the 2-cell stage, which is supported by lineage-tracing experiments[94–96], and by observations that 2-cell embryos divide asynchronously, with daughter cells making a differential contribution to the future ICM or Tr[97,98]. However, the observation that both blastomeres in 2-cell embryos make a similar contribution to all cell types during the postimplantation development of embryos that harbour a *Cre*-reporter indicates that there is no absolute programming of lineage segregation for either the ICM or Tr at the 2-cell stage[90]. This is consistent with studies that used fluorescent-tracer labelling and time-lapse imaging[99–101]. Collective analysis provides evidence that polarity with lineage differentiation is first clearly discernible with the onset of blastocyst formation[101].

Another question in this ongoing debate is whether the first cleavage occurs randomly or is prepatterned[102,103]. It was proposed that the sperm-entry site, along with the second polar body from the meiotic division, marks the first cleavage plane[92,104,105]. However, the results of the labelling of internalized sperm components[106], and the fact that the polar body is not stationary but can move to the cleft between two blastomeres after cleavage[107], challenge this view. Time-lapse recordings show that the first cleavage plane is not predetermined, but is defined by the apposition of two pronuclei at the centre of the zygote[107]. The use of videomicroscopy to visualize the mitotic spindle using GFP-labelled tubulin also shows that the cleavage plane is randomly oriented in 2-cell embryos, arguing against prepatterning before embryonic compaction[108]. Therefore, the question of the origin of cell polarity and lineage differentiation is still under debate.

repression of OCT4 and NANOG in the Tr (FIG. 1b,c). This was shown by the finding that CDX2 deficiency results in a failure to downregulate *Oct4* and *Nanog* in the outer cells of the blastocyst, which results in the loss of the epithelial integrity of these cells and their ultimate demise[23]. Another gene that is involved in Tr development is that encoding the T-box transcription factor EOMES, which, like *Cdx2*, is expressed in the Tr[24]. However, embryos that lack *Eomes* develop to blastocysts and correctly express *Cdx2* and *Oct4* in Tr and ICM cells, respectively[23]. It is suggested that *Cdx2* is the earliest inducer of the Tr lineage in late morulae, whereas *Eomes* is required for Tr proliferation and differentiation at the blastocyst stage.

Determinants of blastocyst competency

For successful implantation to occur in the receptive uterus, the blastocyst must also attain implantation competency. The first evidence that the state of activity of the blastocyst determines the 'window' of implantation in the receptive uterus was derived from reciprocal blastocyst-transfer experiments in a delayed-implantation mouse model[25,26]. This model is a powerful tool for defining the molecular signalling components that direct blastocyst activation or dormancy. Nearly 100 mammals in seven orders undergo delayed implantation[27,28], but the underlying mechanism remains largely unknown. Using this model, a global gene-expression study showed that these two different physiological states of the blastocyst are molecularly distinguishable[29]. The main functional categories of altered genes include cell-cycle, cell-signalling and energy-metabolic pathways. This study also showed an upregulated expression of *Hegf1* (which encodes heparin-binding EGF-like growth factor (HB-EGF)) in activated blastocysts, a finding that is complementary to earlier reports of upregulated expression of its receptors ErbB1 and ErbB4 in similar blastocysts[29–31].

Other signalling molecules also participate in blastocyst dormancy and activation. There is evidence that catecholoestrogens that are produced from primary oestrogens in the uterus activate blastocysts[32]. Another lipid-signalling molecule that targets blastocysts is an endocannabinoid anandamide, which activates G-protein-coupled cannabinoid receptors CB1 and CB2. Expression of *Cb1* in the Tr, and uterine synthesis of anandamide, indicate that endocannabinoid signalling is crucial to implantation in mice[33–35]. Levels of uterine anandamide and blastocyst CB1 are coordinately downregulated with the attainment of uterine receptivity and blastocyst activation, respectively, in contrast to their elevated levels in the nonreceptive uterus and dormant blastocysts[33,36,37]. Indeed, implantation is postponed in wild-type mice that are maintained on sustained levels of exogenously administered cannabinoid ligands, an effect that depends on the expression of CB1 receptors on the embryo[37]. Anandamide regulates blastocyst function by differentially modulating mitogen-activated protein kinase (MAPK) signalling and Ca^{2+}-channel activity via CB1 (REF. 36). This is consistent with findings that MAPK and phosphatidylinositol 3-kinase/Ca^{2+}-signalling cascades are crucial to blastocyst development and activation[38–41].

Most gene-expression studies have so far pointed towards changes in Tr gene expression during blastocyst dormancy or activation. It remains to be seen whether gene expression in the ICM also changes with the state of activity of the blastocyst. A greater insight into the molecular basis of blastocyst competency for implantation might help to improve pregnancy rates in human IVF programs.

Determinants of uterine receptivity

Molecular and genetic evidence indicates that locally produced signalling molecules, including cytokines, growth factors, homeobox transcription factors, lipid mediators and morphogens, together with ovarian hormones, serve as autocrine, paracrine and juxtacrine factors to specify uterine receptivity[2] (TABLES 1,2). In this section, evidence is presented for a novel signalling network that involves cytokines, homeotic proteins and morphogens in implantation.

Cavitation
The creation of a hollow space that appears within the early-cleaving embryos to form a blastocyst.

Embryonic stem cells
(ES cells). Stem cells have the dual capacity to self-replicate and differentiate into several specialized derivatives. ES cells are pluripotent cells that are derived from pre-implantation-stage (usually blastocyst) mammalian embryos. Mouse ES cells can be propagated and manipulated *in vitro*, yet still retain their pluripotency.

Polar body
The structure that is extruded from the oocyte during meiosis, which contains one haploid set of chromosomes.

Window of implantation
A limited time period when the uterine environment is conducive to supporting blastocyst growth, attachment and the subsequent events of implantation.

Delayed implantation
A state of suspended animation of the blastocyst, characterized by halted growth and postponement of implantation. In mice, ovariectomy on day 4 morning of pregnancy, before ovarian oestrogen secretion, initiates blastocyst dormancy, which can last for many days if treated with P_4; an oestrogen injection rapidly activates blastocysts and initiates their implantation.

Blastocyst activation
The event that leads to the competency of the blastocyst to implant.

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Oestrogen and progesterone. The principal hormones that direct uterine receptivity are ovarian P_4 and oestrogen[2]. P_4 is essential for implantation and pregnancy maintenance in all mammals studied, whereas the requirement for ovarian oestrogen is species-specific[2]. For example, ovarian P_4 and oestrogen are essential to implantation in mice and rats, but ovarian oestrogen is dispensable in pigs, guinea pigs, rabbits and hamsters. However, oestrogen that is produced by embryos is considered important for implantation in these last four species[2]; whether ovarian or embryonic oestrogen participates in human implantation remains unknown.

The uterine effects of oestrogen and P_4 are primarily executed by nuclear oestrogen (ER) and progesterone

Table 1 | **Genes implicated in human uterine receptivity for implantation by data from independent microarray analysis**

Gene	Molecule encoded (Putative function)	Comparison of five independent studies				
		LH+(8–10) vs *LH–(4–6)*[60]	*LH+(7–9)* vs *LH+(2–4)*[59]	*LH+7* vs *LH+2*[62]	*LH+(6–8)* vs *LH–(3–5)*[58]	*LH+8* vs *LH+3*[61]
Upregulated						
ANXA4	Annexin-4 (SP)	+		+		+
APOD	Apolipoprotein D (LP)	+	+	+	+	
BNIP2	BCL2/adenovirus E1B 19kDa interacting protein-2 (cell-death protein)		+	+	+	
CLDN4	Claudin-4/CEP-R (R)	+	+	+		
C1R	Complement component-1r (Imm)	+			+	+
*DAF**	Decay accelerating factor for complement (Imm)	+		+	+	+
DF	Complement factor D/Adipsin (Imm)	+		+	+	
DKK1	Dickkopf-1 (WNT antagonist)	+	+	+	+	
GADD45A	Growth arrest and DNA-damage-inducible protein (DNA excision repair, cell-cycle regulator)	+		+	+	+
GBP3	Guanylate-binding protein-2 (GTP-BP)		+	+	+	
ID4	Inhibitor DNA binding-4 (transcription coregulator)	+		+		+
IL15	Interleukin-15 (cytokine)	+	+	+		+
MAP3K5	Mitogen activated protein kinase kinase kinase 5 (MAPK signalling)	+		+	+	+
MT1	Metallothionein-1 family proteins (MBP)	+	+	+	+	+
MAOA	Monoamine oxidase A (catechol-NT metabolizing enzyme)	+		+	+	+
PAEP	Progestagen-associated endometrial protein (SecP)	+		+	+	
SERPING1	Ser (or Cys), clade G (C1 inhibitor), member 1 (proteolysis inhibitor)			+	+	+
SPP1‡	Secreted phosphoprotein-1 (StrP)	+	+	+	+	+
TGFB	TGFβ-super-family proteins	+	+		+	
Downregulated						
CCNB	Cyclin B proteins (cell-cycle regulator)	+	+	+		
FRPHE	Frizzled-related protein frpHE (WNT antagonist)	+	+		+	
GATA2	GATA-binding protein-2	+			+	+
MSX1	Hox Msh-like protein-1	+		+		+
MSX2	Hox Msh-like protein-2	+	+	+		
OLFM1	Olfactomedin-related ER-localized protein-1 (SecP)	+	+	+	+	

Assuming a cycle length of 28–30 days, the surge in the levels of luteinizing hormone (LH) at mid-cycle heralds the onset of ovulation (BOX 2). The comparative levels of global gene expression in the human uterus presented here show that only few genes reveal similar changes (up or downregulation) across five experiments. This poor consistency is perhaps due to changes in the timing of the assay, experimental designs, methods for data analysis, and/or geographical location where subjects reside or the geographical location of the origin of subjects selected. The experiments compare gene-expression profiles between post-LH (+) surge versus pre-LH surge (–) or early versus late post-LH surge. The receptive period in humans spans the period from days 7–10 after the LH surge (LH+(7–10)). Genetic studies in mice might prove fruitful to assess whether these genes are critical to uterine receptivity in humans, since such studies are not possible in humans except for the identification of mutations of these genes in human populations. **DAF*, also known as CD55, Cromer blood-group system. ‡*SPP1*, also known as osteopontin. BP, binding protein; GF, growth factor; Hox, homeobox; Imm, immunomodulator; LP, lipoprotein; MBP, metal-binding protein; NT, neurotransmitter; R, receptor; SecP, secretory protein; SP, signalling protein; StrP, structural protein; TF, transcription factor.

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(PR) receptors. The recent discovery of ER (ERα and ERβ) and PR (PRA and PRB) isoforms and studies of the effect of their selective deletion provide evidence for their isoform-specific functions in uterine biology and implantation. *Erα*$^{-/-}$ uteri are hypoplastic and unable to support implantation[42], whereas *Erβ*$^{-/-}$ uteri retain biological functions that allow normal implantation[2]. Interestingly, P_4 is sufficient for decidualization in *Erα*$^{-/-}$ mice in response to artificial stimuli, which indicates that ERα might be essential for blastocyst attachment, but dispensable for subsequent decidualization[43,44]. The uterus expresses *PRA* and *PRB*. While mice that lack both *PRA* and *PRB* show many defects in ovarian and uterine functions, which leads to female infertility[45], these responses are normal in mice that are missing only *PRB*, which indicates that essential P_4-regulated functions are primarily mediated by *PRA*[46].

Cytokines. Among the cytokines, LIF, a member of the interleukin-6 (IL-6) family, is crucial for uterine preparation for implantation. It binds to the LIF receptor and shares gp130 as a common signal-transduction partner with other cytokines. In mice, *Lif* is expressed first in uterine glands on the morning of day 4, and then in stromal cells that surround the blastocyst during attachment[47,48]. This indicates that LIF has a dual role: initially in uterine preparation and later in attachment. Blastocysts remain 'dormant' in *Lif*$^{-/-}$ mice and do not implant, an effect that depends on the uterine LIF mutant status[47,48]. The molecular mechanism by which LIF executes its effects on implantation is still unclear, although inactivation of the gp130 protein by deleting its signal transducer and activator of transcription (STAT) binding sites also results in implantation failure[49]. Uterine *Lif* expression is high around the time of implantation in other species, including humans[2].

Homeobox proteins. Several homeobox transcription factors are crucial to uterine receptivity and implantation. In mice, two *Abdominal-B*-like *Hox* genes, *Hoxa10* and *Hoxa11*, are expressed in uterine stromal cells during the receptive phase. This expression persists during postimplantation decidualization, which might indicates an overlapping role for the two genes in uterine receptivity, implantation and decidualization[50–53]. Most *Hoxa10*$^{-/-}$ females are infertile, primarily due to a reduced stromal-cell proliferation and the consequent failure to decidualize[50,52]. However, *Hoxa10* does not seem crucial for uterine receptivity, because initial uterine attachment of blastocysts can occur in *Hoxa10*$^{-/-}$ mice, and *Hoxa10* expression is normal in *Lif*$^{-/-}$ uteri[54]. In contrast, *Hoxa11*$^{-/-}$ uteri are hypoplastic, have fewer glands and show a more severe phenotype than *Hoxa10*$^{-/-}$ mice[55]. More importantly, the absence of *Lif* expression in *Hoxa11*$^{-/-}$ uteri indicates that *Hoxa11* might be crucial to uterine receptivity and later events of implantation[55]. Both *Hoxa10* and *Hoxa11* are upregulated in the human uterus during the secretory phase, which indicates that they might have a role in uterine receptivity[56]. Gene-targeting experiments show that blastocysts fail to implant in *Hmx3*$^{-/-}$ mice, but the reason for this failure remains unknown because *Hmx3*, which belongs to a different homeobox gene family to *Hox* genes, is mainly expressed in the myometrium[57].

Another homeobox gene, *Msx1*, is transiently expressed in the mouse uterine epithelium during the receptive period, but disappears at the time of blastocyst attachment or when the uterus enters the nonreceptive phase[54]. Sustained expression of *Msx1* in *Lif*$^{-/-}$ mice further reinforces the importance of *Msx1* in uterine receptivity. It is interesting that *Msx1* is downregulated in the receptive human endometrium[58–62] (TABLE 1). However, a definitive role for *Msx1* in uterine receptivity requires conditional uterine deletion, because offspring that are missing *Msx1* die shortly after birth due to craniofacial defects[63].

Morphogens. One less-explored area is the role of morphogens in uterine receptivity and implantation. Embryo–uterine interactions during implantation share many features with reciprocal epithelial–mesenchymal interactions during embryogenesis, and both involve evolutionarily conserved signalling pathways. The importance of hedgehog (HH), WNT and bone-morphogenetic-protein (BMP) signalling in uterine receptivity was recently explored. The genes encoding the components of the HH signalling pathway, namely Indian hedgehog (*Ihh*), HH-binding protein/receptor Patched (*Ptc*) and the transcription factors *Gli1–3* are expressed in the mouse uterus[64,65]. *Ihh* expression is P_4-dependent and reaches high levels in epithelial cells on day 4, while that of *Ptc*, *Gli1* and *Gli2* is upregulated in the underlying stroma. In day 4 uterine-explant cultures, recombinant N-sonic hedgehog (N-SHH) stimulates mesenchymal-cell proliferation, a characteristic of the receptive phase[65]. These findings indicate that epithelial IHH functions as a paracrine growth factor for stromal cells and that this epithelial–mesenchymal signalling is important for uterine receptivity.

The roles of WNT and BMP signalling in preserving tissue boundaries in the adult uterus remain largely unknown. *sfrp4*, a WNT antagonist and a member of the secreted Frizzled-related proteins (sFRPs), and *Noggin*, an anti-BMP, are expressed in the uterine stroma during the receptive phase[66]. *Wnt4* and *Bmp2* are not expressed at this time, but are induced in the stroma with the onset of blastocyst attachment, and thereafter with disappearing expression of the antagonists[54,66]. These findings indicate that while HH signalling participates in uterine receptivity, WNT4 and BMP2 are involved in the attachment reaction and postimplantation events.

Why, then, are *sfrp4* and *Noggin* expressed in the absence of their ligands? Do they have functions that are independent of their ligands? Are other members of the ligand family expressed in the uterus? A marked downregulation of *sfrp4* in *Lif*$^{-/-}$ uteri indicates that a WNT-signalling component is important in uterine preparation[54]. Alternatively, this downregulation might be a consequence of compromised uterine function in the absence of *Lif*. Of the WNT family, *Wnt7a* is expressed in the LE in adult females, and deletion of the *Wnt7a* gene shows global posterior shifting of the

Hypoplastic
Refers to an underdeveloped tissue or organ.

Decidualization
Transformation of stromal cells into morphologically and functionally distinct cells. Part of decidualized tissue is shed at parturition.

Attachment
A process by which the blastocyst trophectoderm is brought into physical and physiological contact with the uterine luminal epithelium.

Myometrium
The muscular outer layer of the uterus, which is comprised of longitudinal and circular muscle fibers.

Endometrium
The inner lining of the uterus; it is primarily comprised of stromal cells (the supporting tissue of an organ) and epithelial cells of both luminal and glandular types. Part of the endometrium is shed during menstruation.

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Table 2 | **Genes critical to uterine biology and implantation: results of mouse knockout models**

Genes	Molecule encoded (Putative function)	HomoloGene No. (NCBI)	Knockout phenotype in females	Refs
Uterine patterning during postnatal growth				
Hoxa10	Homeobox A10 (TF)	7365	Homeotic transformation of anterior uterus to oviduct	50,53, 114
Hoxa11	Homeobox A11 (TF)	4033	No uterine glands; partial homeotic transformation of uterus to oviduct	51
Wnt5a	WNT-5a protein (SP)	20720	No morphologically defined cervix; no uterine glands	115
Wnt7a	WNT-7a protein (SP)	20969	Abnormal oviduct and uterine development*; infertility	67
Uterine physiology in adult life				
Adamts1	A disintegrin-like and metalloprotease with thrombospondin-type-1 motif-1 (Enzyme, tissue remodelling	21381	Impaired follicular development and fertilization; uterine cysts; subfertility	116
Bteb1	Basic transcription-element-binding protein-1 (TF)	931, 79195	Uterine hypoplasia; compromised uterine P_4 function; impaired embryo implantation; subfertility (ME)	117
Cenpb	Centromere protein B (Centromere assembly)	1370	Disrupted luminal and glandular uterine epithelia; subfertility (genetic-background-dependent)	118
Cyp27b1	25-hydroxyvitamin D 1α–hydroxylase enzyme (Vitamin D metabolism)	37139	Uterine hypoplasia; absence of corpus luteum; infertility	119
Esr1	Oestrogen receptor-α (NR,TF)	47906	Ovarian cysts; uterine hypoplasia; infertility	42
Igf1	Insulin-like growth factor-1 (GF)	515	Ovulation failure; uterine myometrial hypoplasia; infertility	120
Pgr	Progesterone receptor (NR,TF)	713	Unopposed oestrogen action; uterine hyperplasia; infertility	45
Ube3a‡	Ubiquitin-protein ligase E3A (Protein modification, proteolysis and peptidolysis)	7988	Impaired follicular development and uterine hypoplasia; subfertility	121
Vdr	Vitamin D receptor (R,TF)	37297	Uterine hypoplasia with impaired folliculogenesis; infertility	122
Uterine preparation for initiating implantation				
Bsg	Basigin (Immunoglobulin)	1308, 45225	Defective fertilization; no implantation	123,124
Esr1	Oestrogen receptor-α (NR,TF)	47906	No uterine attachment, but uterine responsiveness to decidualization persists with P_4 priming	42,44
Fkbp52	FK506-binding protein-4 (Immunophilin co-chaperone for steroid hormone NRs)	36085, 43060	Compromised P_4 function; no uterine receptivity (ME)	69
Gp130/ Stat	GP130/Signal Ttransducer and activator of transcription (Cytokine-receptor signalling)	1645	No implantation (ME)	49
Hmx3	H6 homeobox-3 (TF)	40612	No implantation (ME)	57
LpA3	Lysophosphatidic acid receptor-3 (LPA signalling)	8123	Deferred, on-time implantation; aberrant embryo spacing; postimplantation defects; small litter size (ME)	80
Lif	Leukaemia inhibitory factor (Cytokine)	1734	No implantation (ME)	48
Pgr	Progesterone receptor (NR,TF)	713	No implantation or decidualization (ME)	45
Pla2g4a	Phospholipase A2, group IVA§ (Arachidonic-acid-releasing enzyme)	32059	Deferred on-time implantation; aberrant embryo spacing; postimplantation defects; small litter size (ME)	79
Ppard	Peroxisome proliferator-activated receptor-δ (NR,TF)	4544	4–6 h delay in initiating embryo attachment; placental defects; subfertility	76,125
Ptgs2¶	Prostaglandin-endoperoxide synthase-2 (Prostaglandin synthesis)	31000	Multiple reproductive failures, including defective attachment reaction; genetic-background-dependent	75,77
Uterine decidualization				
Fkbp52	FK506-binding protein-4 (Immunophilin co-chaperone for steroid hormone NRs)	36085, 43060	Compromised P_4 function; defective decidualization (ME)	69
Hoxa10	Homeobox A10 (TF)	7365	Defective decidualization; reduced fertility (ME)	50,52,53
Hoxa11	Homeobox A11 (TF)	4033	Defective implantation and decidualization; infertility	51
Il11ra1	Interleukin-11 receptor-α1 (Cytokine signalling)	3316	Impaired decidualization; infertility	126,127
Pgr	Progesterone receptor (NR,TF)	713	Lack of decidual response even after P_4 priming	45
Ptgs2¶	Prostaglandin-endoperoxide synthase-2 (Prostaglandin synthesis)	31000	Defective decidualization; reduced angiogenic response	75,85

*Also, reduced stromal tissue, lack of uterine glands and disorganized myometrium. ‡*Ube3a*, also known as E6AP ubiquitin-protein ligase. §Cytosolic, calcium-dependent. ¶Also known as cyclooxygenase-2 (COX2). GF, growth factor; ME, maternal effect; NR, nuclear receptor; R, receptor, SP, signalling protein; TF, transcription factor.

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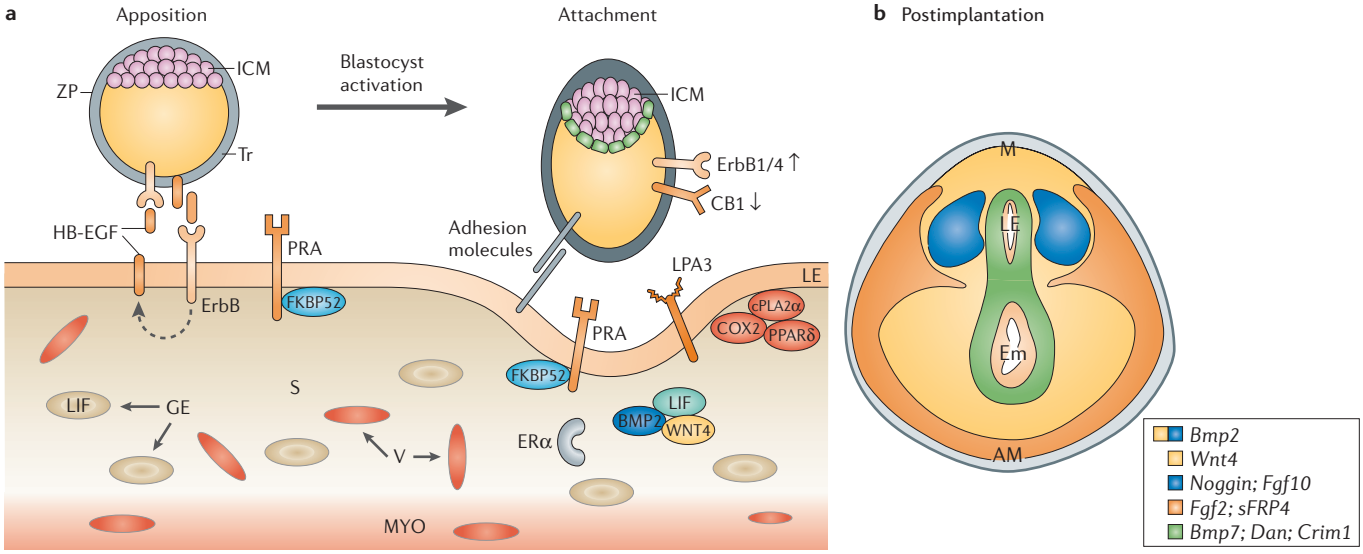

Figure 2 | **Gene products participating in embryo implantation. a** | Signalling pathways that are known to coordinate blastocyst apposition and attachment in the mouse uterus. Apposition and attachment are key steps in implantation and absolutely depend on the synchronized development of the blastocyst to implantation competency and differentiation of the uterus to the receptive stage. Ovarian oestrogen and progesterone, acting through their cognate nuclear receptors, influence several locally produced growth factors, adhesion molecules, cytokines, transcription factors and vasoactive mediators and their receptors in the uterus and/or blastocyst to coordinate blastocyst–uterine crosstalk. This crosstalk further influences some of the signalling pathways to ensure the successful execution of the implantation process. **b** | Region-specific expression patterns of morphogens in the mouse deciduum during the postimplantation period. This scheme is based on *in situ* hybridization of the indicated genes in a representative cross-section of an implantation chamber on day 7 of pregnancy. AM, antimesometrial pole; BMP2, bone morphogenetic protein-2; CB1, brain-type cannabinoid receptor-1; COX2, cyclooxygenase-2; cPLA2α, cytosolic phospholipase $A_{2\alpha}$; Crim1, cysteine-rich transmembrane BMP-regulator-1; Dan, differential screening-selected gene aberrative in neuroblastoma; Em, embryo; ERα, nuclear oestrogen receptor-α; ErbB, EGF-receptor family; FGF, fibroblast growth factor; FKBP52, FK506 binding protein-4; GE, glandular epithelium; HB-EGF, heparin-binding EGF-like growth factor; ICM, inner cell mass; LE, luminal epithelium; LIF, leukaemia inhibitory factor; LPA3, lysophosphatidic-acid receptor-3; M, mesometrial pole; MYO, myometrium; PPARδ, peroxisome-proliferator-activated receptor-δ; PRA; nuclear progesterone receptor A; S, stroma; sFRP4, secreted Frizzled-related protein-4; Tr, trophectoderm; V, blood vessels; ZP, zona pellucida.

reproductive tract, with the loss of *Hoxa10* and *Hoxa11*. *Wnt7a*$^{-/-}$ females are infertile, with uteri that lack glands and disorganized myometria, which indicates that *Wnt7a* might be crucial for normal uterine cellular architecture[67]. Of the *Bmp* genes that have been studied, *Bmp4–7* and *8a* do not show the same highly localized expression pattern as *Bmp2* during attachment[66]. An investigation that spans other members of the WNT and BMP families, their receptors and putative antagonists is warranted to better understand the roles of these morphogens in uterine receptivity.

Signalling during implantation

The process of implantation is classified into three stages: apposition, attachment (adhesion) and penetration. During apposition, the Tr becomes closely apposed to the LE. This is followed by the attachment stage, when the association of the Tr and LE is sufficiently intimate to resist dislodging of the blastocyst by flushing the uterine lumen. The first sign of the attachment reaction occurs on the evening of day 4 in mice, and coincides with a localized increase in stromal vascular permeability at the site of blastocyst attachment. Penetration involves invasion of the embryo through the LE and basal lamina into the stroma, to establish a vascular relationship with the mother. At this stage, stromal-cell differentiation into decidual cells (decidualization) is extensive and leads to the loss of the LE at the site of the implanting blastocyst. (Note, however, that stromal-cell decidualization also occurs in women during the luteal phase of the menstrual cycle, in the absence of an embryo.) The dynamic and overlapping expression of signalling molecules during these three stages makes it difficult to assign the contribution of specific signalling pathways to a particular stage (FIG. 2; TABLE 2).

Apposition. In rodents, a generalized stromal oedema leads to uterine luminal closure, resulting in interdigitation of microvilli of the Tr and LE (apposition). Luminal closure occurs in pregnant or pseudopregnant uteri, and therefore does not require the presence of blastocysts. P_4 priming, however, is essential for closure. This is supported by the absence of luminal closure in pregnant mice that are missing FK506 binding protein-4 (FKBP52), a co-chaperone that is required for appropriate uterine PR function[68,69]. *Fkbp52* expression overlaps with that of PR in the stroma before the attachment reaction, and *Fkbp52*$^{-/-}$ females show implantation failure and

Basal lamina
A thin sheet of proteoglycans and glycoproteins that are secreted by cells as an extracellular matrix. It is also called the basement membrane and influences cell polarity, differentiation and migration.

Decidual cells
In the mouse, the cells that surround the implanting blastocyst.

Oedema
Fluid accumulation in the intercellular tissue spaces.

Luminal closure
The closure of the uterine lumen, resulting in closer contact between the luminal epithelial linings; this step is essential for blastocyst attachment.

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downregulation of the P_4-responsive genes *Areg* (which encodes amphiregulin), *Hoxa10* and *Ihh* in the uterus. However, although P_4 priming via PR is essential for luminal closure and apposition, blastocyst attachment cannot occur unless the P_4-primed uterus is exposed to oestrogen.

The signalling pathway that is initiated by HB-EGF has been studied extensively during apposition and attachment because HB-EGF is an early molecular marker of embryo–uterine crosstalk[70]. *Hegf1* is expressed in the mouse LE at the site of blastocyst apposition several hours before attachment, and this persists through the early attachment phase. HB-EGF is produced as soluble and transmembrane forms. Molecular and genetic evidence show that it influences embryonic functions as a paracrine and/or juxtacrine factor by interacting with ErbB1 and/or ErbB4, which are expressed on the blastocyst cell surface[30,31,38]. Most *Hegf1*$^{-/-}$ mice die during prenatal and early postnatal life due to cardiac defects[71], precluding an examination of the implantation phenotype.

Implantation-competent blastocysts that also express *Hegf1* induce expression of the gene in the uterus in a paracrine manner[29]. This auto-induction loop is perhaps the first example of molecular crosstalk between the blastocyst and uterus, initiating the attachment reaction. HB-EGF also has a role in human implantation. Its expression is maximal in the receptive endometrium, and cells that express transmembrane HB-EGF adhere to blastocysts that display cell-surface ErbB4 (REF. 72).

Attachment. It is correctly assumed that adhesive-signalling systems are required for the attachment phase. Indeed, numerous glycoproteins and carbohydrate ligands and their receptors are expressed in LE and Tr cells around the time of implantation. The most important adhesion molecules that are implicated in this process are integrins, selectins, galectins, heparan sulfate proteoglycans (HSPGs), mucin-1, cadherins and the trophinin–tastin–bystin complex[1,2,4]. Integrins and selectins are of special interest because of their unique functional features. In the human uterus, integrin αvβ3 is localized to the LE during the receptive phase, and its aberrant expression is correlated with infertility and recurrent pregnancy loss[1]. Recent evidence shows that selectin signalling is also important in human implantation. While selectin oligosaccharide ligands are expressed in the receptive LE, L-selectin molecules are displayed on the Tr cell surface[73]. More importantly, beads that are coated with specific selectin ligands adhere to trophoblast cells and, conversely, isolated trophoblast cells bind preferentially to the receptive uterine surface. These findings indicate that the selectin-adhesion system constitutes an initial step in human implantation. However, apparently normal fertility in mice that lack L-selectin indicates a species-specific variation in the adhesion cascade during implantation. As stated before, *Lif* also seems to be important for the attachment process, because *Lif*$^{-/-}$ mice show a lack of HB-EGF and aberrant cyclooxygenase-2 (*Cox2*) expression in blastocysts during the anticipated time of attachment[47,74].

Penetration. One key event in implantation is an increased endometrial vascular permeability at the site of blastocyst attachment and penetration (FIG. 2), a process that involves the action of prostaglandins (PGs). COX1 and COX2 mediate PG synthesis and are encoded by *Ptgs1* and *Ptgs2*, respectively. *Ptgs2* expression is unique in the mouse uterus, and shows expression in the LE and underlying stromal cells at the site of blastocyst attachment[75]. It is speculated that HB-EGF that is produced in the uterus and embryo induces uterine *Ptgs2* expression. *Ptgs2*$^{-/-}$ females are largely infertile, with defective ovulation, fertilization, implantation and decidualization[75]. COX2-derived prostacyclin (PGI_2) is the primary PG that is produced at the implantation site, and implantation defects are improved in *Ptgs2*$^{-/-}$ mice by PG administration[76]. Evidence indicates that PGI_2 participates in implantation via the activation of peroxisome-proliferator-activated receptor-δ (PPARδ), the expression of which overlaps with *Ptgs2* at the implantation site[76]. However, depending on the genetic background, COX1 can compensate for COX2 to improve infertility in *Ptgs2*$^{-/-}$ females[77]. *Cox2* is also expressed in the uterus and/or blastocyst during implantation in several species, including primates[78], indicating a conserved function for COX2 in implantation.

The function of PG is further illustrated by the reduced fertility of mice that lack cytoplasmic phospholipase $A_{2\alpha}$ (cPLA2α), which generates a precursor for PG synthesis. Compromised fertility is due to the deferral of on-time implantation, which leads to inappropriate embryo spacing, retarded feto-placental development and reduced litter size[79]. These results reveal that the cPLA2α–COX2 signalling axis is crucial to implantation. Signalling by lysophosphatidic acid (LPA), which belongs to a lysophospholipid group, also influences blastocyst attachment in mice by activating the G-protein-coupled receptor LPA3 (REF. 80). Like the *cPla2α*$^{-/-}$ mice, *lpA3*$^{-/-}$ females show deferred implantation and its associated defects. The treatment of both *cPla2α*$^{-/-}$ and *lpA3*$^{-/-}$ mice with PGs resumes on-time implantation, but embryo crowding persists. Phenotypic similarities between *lpA3*- and *cPla2α*-deficient mice and reduced levels of uterine COX2 in *lpA3*$^{-/-}$ mice identify COX2 as a common signalling pathway.

Implications for human fertility. From the results discussed above, one important finding is that a short delay in blastocyst attachment creates an adverse ripple effect throughout the course of pregnancy, which leads to defective feto–placental development and poor pregnancy outcome. This indicates a new concept in which embryo–uterine interactions during implantation set up subsequent developmental programming. This idea is supported by the clinical finding that implantation beyond the normal window of receptivity is associated with a higher risk of early pregnancy loss in women[81]. The downstream pathways of PG signalling that participate in the ripple effect remain unknown. In light of these findings, one can assume that many previous studies that describe early

Integrins
A family of receptors for various extracellular-matrix ligands that modulate cell–cell adhesion and signal transduction. Each integrin has two subunits, α and β, and each αβ combination has a unique binding specificity and unique signalling properties.

Selectins
A group of cell-adhesion molecules, including L-selectin, E-selectin and P-selectin, that bind to carbohydrates.

Galectins
A family of lectins with galactose-binding ability.

Trophinin–tastin–bystin complex
A homophilic cell-adhesion complex that is comprised of membrane–cytoplasmic proteins.

Prostaglandins
(PG). Vasoactive lipid mediators that are implicated in various pathophysiological processes, including vascular permeability, angiogenesis and cell migration.

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or mid-gestational embryonic lethality arising from specific mutations might have originated at the time of implantation.

Another unresolved issue is embryo spacing in the uterus. While the LPA3–cPLA2α–COX2 signalling axis is important for normal embryo spacing, attachment and penetration, no molecule has been found to rescue the spacing defect in mice that are mutant for the processes involved. Answers to the question of how embryo spacing is regulated might provide insights into the aetiology of placenta previa in humans. Is it possible that embryo spacing is regulated by local factors that are associated with PG signalling? BMPs are required for the spacing of tissue structures during development[82], and local delivery of BMP2 or BMP4 in the uterus causes aberrant embryo spacing[66]. There is also genetic evidence that BMP5 and NODAL are important for this process[83]. As there is a relationship between BMP and PG signalling in other systems[84], these pathways might work together to influence embryo spacing.

Postimplantation uterine development

Uterine stromal cells that surround the blastocyst undergo decidualization following attachment, eventually embedding the embryo in the antimesometrial stromal bed. One function of the deciduum is to provide nutritional support to the developing embryo before the establishment of a functional placenta. Numerous signalling molecules, including cytokines, homeobox transcription factors, cell-cycle molecules, extracellular-matrix remodelling factors and lipid mediators, are expressed in the endometrium during decidualization and are crucial to this process[2]. Here, we focus on the less-explored areas, such as uterine angiogenesis and establishment of the uterine–embryonic axis during the postimplantation period (FIG. 2b).

Uterine angiogenesis. Under physiological conditions in adult females, angiogenesis primarily occurs in the uterus and ovary during the reproductive cycle and pregnancy. Angiogenesis is essential to normal implantation and placentation, and is profoundly influenced by vascular endothelial growth factor (VEGF) and angiopoietins. PGs, because of their role in angiogenesis in other systems, are also thought to participate in uterine angiogenesis during pregnancy. But what is the link between VEGF, angiopoietin and PG signalling? The VEGF receptor FLK1 (also known as KDR, kinase-insert-domain protein receptor) is a marker of endothelial cells during angiogenesis. Using *Ptgs2*$^{-/-}$ x *Flk1*$^{+/-LacZ}$ reporter mice, it was shown that COX2-derived PGs markedly influence uterine angiogenesis during decidualization by differentially regulating VEGF and angiopoietin signalling cascades[85]. Uterine angiogenesis in *Ptgs2*$^{-/-}$ mice is severely compromised, owing to defective VEGF, but not angiopoietin, signalling and this defect is rescued by exogenous PG. Because PGs coordinate VEGF signalling with that of angiopoietins during decidual angiogenesis, one cause of compromised implantation and decidualization in *Ptgs2*$^{-/-}$ mice could be dysregulated vascular events.

Establishment of the uterine–embryonic axis. The adult uterus undergoes dynamic cellular and molecular changes during pregnancy, but how these changes are coordinated to specify the allocation of new cell types, for example, decidual cells and their boundaries, remains largely unknown. Decidualization is initiated at the antimesometrial pole, subsequently extending to the mesometrial pole, the presumptive site of placentation. This orients the implantation chamber in an antimesometrial–mesometrial (AM–M) direction, in alignment with the embryonic axis. It is still unclear how the implantation chamber is oriented and grows in an AM–M direction, with the decidual reaction spreading in the same direction. It is also not known how decidual cell growth is restricted, leaving a layer of undifferentiated stromal cells underneath the myometrium.

It is speculated that WNT signalling, in collaboration with those of BMP and fibroblast growth factor (FGF), helps to orient the implantation chamber in the AM–M direction and specifies these boundaries during decidualization (FIG. 2b); in particular, differential WNT4 signalling seems to participate in making this boundary[54]. An inverse relationship with respect to *Bmp2* and *Noggin* expression that is observed during implantation and decidualization also indicates differential BMP signalling during early pregnancy[66]. However, the expression of *Dan* (differential screening-selected gene aberrative in neuroblastoma), a member of the *Dan/Dante Bmp*-antagonist gene family, and *Crim1* (cysteine-rich transmembrane BMP-regulator-1), which encodes a protein that is thought to bind BMP, partially overlap with that of *Bmp2* expression. Furthermore, antimesometrial expression of *Fgf2*, in contrast to mesometrial expression of *Fgf10*, adds to evidence that the AM–M orientation of the uterus during early pregnancy is influenced by differential gene expression[66]. We speculate that uterine orientation helps to establish embryonic orientation during development, and that the failure of the implantation chamber to orient itself in an AM–M direction is likely to disrupt embryonic orientation. Therefore, these developmental genes are not only important for establishing boundaries and polarities during embryogenesis, but also for establishing the orientation of the growing implantation chamber and creating boundaries to prevent undifferentiated stromal cells from decidualizing (the undifferentiated stromal cells might serve to replenish the stroma after parturition).

How mice can help humans

Studies in mice have provided insights into the molecular basis of human implantation because of their shared features. Both mouse and human embryos can develop *in vitro* in simple, defined media. In both species, embryo implantation leads to stromal decidualization — embryos embed in the antimesometrial stroma and placentation is hemochorial.

Mouse embryos grow more effectively when they are cultured in a small volume[86]. This protocol, which is practiced by some clinics, has shown improved embryo

Placenta previa
A condition in humans in which the placenta is situated close to or covering the cervix.

Hemochorial placentation
The process by which maternal blood comes in direct contact with the trophoblast.

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Box 4 | **Future challenges for reproductive biology and reproductive medicine**

- There is a need to identify reliable markers of uterine receptivity and to develop the means to extend uterine receptivity or treat nonreceptivity to improve the pregnancy rate in *in vitro* fertilization and embryo-transfer programmes. Overcoming these challenges will lessen the need to transfer multiple embryos to increase the pregnancy rate and the resulting complications of multiple pregnancies.
- Although various signalling pathways operate during implantation, it is still unclear whether they work independently, in parallel or converge on a common pathway.
- Suitable animal models must continue to be developed to define the molecular communication between the uterus and embryo. Such studies require information about the contribution that is made by each of the two tissues, a task not easily achievable in humans because of experimental difficulties and ethical restrictions on research with human embryos.
- Another challenge is to identify gene promoters for creating inducible *Cre*-transgenic mice for conditional gene deletion specifically in uterine cells; genome-wide deletion of many implantation-associated genes leads to embryonic lethality, precluding studies on implantation. This approach will also help to elucidate the long-term versus acute effects of a gene during implantation. This is particularly important in the context of the adaptation of animals to a new make-up. For example, deletion of one gene that does not affect pregnancy under normal conditions shows adverse effects under a stress situation[109].
- Efforts should continue in establishing a relevant *in vitro* model of implantation to study the hierarchy of events that are triggered by the embryo, and the function of specific signalling molecules.
- There is a need to properly annotate the markers of uterine receptivity that are derived from microarray experiments in rodents and humans[58–62,110,111] with results from functional analyses. Comparative proteomics between wild-type and mutant uteri (an assay that is under-exploited in the field) should provide unique information[68].
- Another approach that shows great promise is the direct analysis of tissue sections by MALDI mass spectrometry to identify the spatial localization of proteins[112]. This approach is attractive for comparing proteome profiles of implantation versus interimplantation sites or between different regions within an implantation site.
- The nature of embryonic signals that influence uterine functions is mostly unknown. In rodents and humans, the limiting factor is the availability of adequate amounts of tissues for analysis. With the advent of microscale proteomics and genomics, it might now be possible to identify embryonic signals during implantation. Once potential molecules are identified, their functions could be assessed by local application into the uterus using blastocyst-sized beads as carriers[66].

development in human IVF programs. However, the pregnancy success rate remains poor (~30%) due to the transfer of IVF-derived embryos into nonreceptive uteri. One cause of nonreceptivity might be high oestrogen levels; this results from the gonadotropin treatment that is given to women to stimulate the ovaries to produce multiple eggs. Indeed, the range of oestrogen levels that determines uterine receptivity in mice is narrow[26]. This remarkable uterine sensitivity to oestrogen might be important to ensure the correct timing of implantation, which is crucial to pregnancy outcome. Understanding the cause of uterine nonreceptivity at higher oestrogen levels might make it possible to extend uterine receptivity by using an aromatase inhibitor to neutralize excess oestrogen during gonadotropin stimulation.

Early onset of intrauterine growth restriction, recurrent abortion, preeclampsia and preterm delivery are important reproductive health issues, and are associated with placental deficiencies. A transient postponement of blastocyst attachment in mice produces a detrimental ripple effect throughout pregnancy, which indicates that these end results are due to defective implantation. Such defects could be corrected, because signalling by LIF, HB-EGF, COX2 and HOX family members, which are important at different stages of implantation in mice, are also thought to be important for human implantation. In fact, downregulation of HB-EGF expression in the human trophoblast is associated with preeclampsia[87]. Further insights into these and the recently identified pathways that are described above might improve pregnancy success and could also help in designing new and improved contraceptives. There is a need to develop nonsteroidal contraceptives so that women are spared the complications of hormonal imbalances and the risk of developing gynecological cancers. Molecular approaches to disrupt LIF–STAT, FKBP52 or LPA3–COX2 signalling pathways might be considered for potential contraceptives.

Conclusion

Implantation is an incredibly useful biological system, a better understanding of which will advance our knowledge in several basic physiological processes. These include: the paracrine and juxtacrine epithelial–epithelial interactions that occur between the Tr and LE during attachment; the epithelial–mesenchymal interaction between the LE and the stroma; Tr–epithelium–stroma interactions, involving cell migration and invasion; vascular permeability and adult angiogenesis; and regulated growth (proliferation, differentiation, polyploidy and apoptosis) during stromal decidualization. Despite the large strides that have been made by applying genomics and proteomic approaches to rodents, the field faces many important challenges (BOX 4).

Implantation involves numerous signalling pathways that are common to other systems under either normal or pathological conditions. Therefore, research on implantation should appeal to a broader range of scientists, not solely to reproductive or developmental biologists. For example, many of the features and signalling pathways that are required during implantation are also active during tumourigenesis — the difference being that tight

MALDI mass spectrometry
(Matrix-assisted laser desorption/ionization mass spectrometry). It is based on the co-crystallization of a test compound with an ultraviolet-light-absorbing matrix, which allows ionization using laser excitation to determine the mass of the test compound.

Preeclampsia
The development of hypertension with proteinuria (excess protein in urine) and/or oedema during pregnancy; early onset occurs from defective trophoblast function.

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regulation occurs during implantation, while dysregulation of the same pathways occurs in tumourigenesis. Therefore, understanding the intricacies of implantation might help to better understand the complexities of tumourigenesis, and might one day reveal that 'life and death are linked by a common thread'.

Note added in proof

The discoveries that CDX2 and OCT3/4 are crucial for specifying Tr and ICM cells, respectively, in pre-implantation embryos have now been expanded to show their roles in stem-cell biology. A report shows that embryos that are missing CDX2 develop to blastocysts, but fail to implant because they lack Tr. These blastocysts have ICMs and generate ES cells when grown in culture[128]. Another recent report shows that ES cells can be forced to differentiate to trophoblast stem (TS) cells by repressing OCT3/4 (REF. 129). This study shows that CDX2 is dispensable for Tr differentiation, but is required for TS-cell self renewal, which indicates that the reciprocal inhibition of transcription factors OCT3/4 and CDX2 participates in lineage differentiation in mammalian embryos[129]. Although the cell-lineage specification in mammalian embryos is primarily thought to occur between the 8-cell and blastocyst stages, the question of how embryonic polarity is established is still a subject of debate. Gore *et al.*[130] now show that maternal *Squint*, a mammalian NODAL-related morphogen, localizes to two blastomeres at the 4-cell stage and specifies the dorsal axis, which indicates that NODAL could also be involved in determining polarity in early mammalian embryos.

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Competing interests statement

The authors declare no competing financial interests.

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