ENDOMETRIAL RECEPTIVITY: THE JOURNEY SO FAR

[1]K Osazee and [2]A Omorogiuwa

[1]Department of Obstetrics and Gynaecology, School of Medicine, College of Medical Sciences, University of Benin, Nigeria.
[2]Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin City, Benin City, Nigeria

ABSTRACT

Implantation is an act of coordinated interaction between the nascent blastocyst and a receptive endometrium mediated by the molecular and cellular interplay in a spatiotemporal manner. Though the advent of modern technology has enhanced the availability of relatively good quality embryos; the implantation rate has not positively correlated. Unlike the human embryo, the study of implantation is laden with ethical and technical challenges. Hence, most of the data on the process of implantation derived from animal studies. Unfortunately, there is wide variation in implantation process among animal species. Thus, cannot be transposed for a human. Hence, the in-vivo model remained the basis for the study of the mechanism of implantation. Research directed towards this direction may help in optimising the outcome of Assisted Reproduction Technology (ART).

KEYWORDS: Endometrial receptivity, Blastocyst, Implantation, Infertility.

INTRODUCTION

Implantation is the process of complementary interaction of the endometrium and the nascent blastocyst often achieved in a stepwise fashion of apposition, adhesion, and invasion predicated on genetic and cellular signals[1]. Though, the viability of the embryo is essential, the receptivity of the endometrium has been shown to be pivotal given its propensity to create a barrier to implanting blastocyst[2]. For optimal receptivity, there must be synchronous molecular and cellular interplay between the endometrium and the blastocyst guaranteed by the ovarian steroid-primed endometrium within a time frame in the mid-secretory phase termed Window of Implantation (WOI)[3]. An attempt at synchronizing embryo transfer within the period of optimal endometrial receptivity has led to several types of research aim at improving the success rate of ART as well as unravel the causes of unexplained infertility and recurrent implantation failure (RIF). Unfortunately, there is no consensus on the biomarker to establish it[4].

Success has been made in the study of human embryos in- vitro. Unfortunately, the uterus is not accessible to demonstrate the exact site of implantation for technical and ethical reasons. The situation is even compounded by the heterogeneity associated with implantation process in different species[5]. Thus, make it difficult to develop the right animal model for research. Hence, the in-vivo model has remained the basis for the study of the physiological and pathological mechanism of implantation.

MENSTRUAL CYCLE

Menstrual cycle involves series of organized events comprising the hypothalamus, anterior pituitary, Ovary, and endometrium. Commonly referred to as the hypothalamic pituitary ovarian axis[6]. The morphological and physiological modifications involved in these organs in the course of the menstrual cycle are subject to the autocrine, paracrine and endocrine effect associated with the axis[7]. At the onset of the cycle, the gonadotropins are secreted under the influence of hypothalamus, by the anterior pituitary. The hormone impact on the ovary to secrete the steroid hormones (estrogen, progesterone and other peptides) in the course of folliculogenesis[8]. These steroid hormones are

Corresponding Author: DR OSAZEE KEHINDE
Department of Obstetrics and Gynaecology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.
E-mail: kehindeosazee@gmail.com. Tel: +2348034024470

responsible for the structural and functional changes associated with the endometrium in anticipation for conception or menstruation to mark the beginning of another cycle in the absence of pregnancy[6].

The dynamics of the endometrium is initiated by the estrogen secretion resulting in increase production and stimulation of estrogen receptor alpha and progesterone receptor isoforms. Thus triggers the expression of relevant genes and cell division and proliferation in what is term proliferative phase of the endometrium[9,10]. The phase is characterized by the hypertrophy of the stroma and glandular cells as well as the elongation of the spiral vessels [6]. Following ovulation, the progesterone is secretion by the corpus luteum. This terminates the proliferative process through the disappearance of the estrogen receptor alpha[11] and heralds the onset of the early secretory phase of the endometrium. The phase is characterized by the secretion of mucus and glycogen from the glandular cells[12]. In the mid-secretory phase, the progesterone, through its receptor acts on the stroma tissue. Thus, makes the stromal cells render paracrine function by stimulating the expression of epithelial genes necessary for the implantation of the embryo[9]. In light of the associated decidualization and other respective potentials for implantation, the phase is often related to the window of implantation[7].

The period termed WOI is characterized by epithelial luminal transformation and changes for the trophoblast attachment and apposition[13,14] and associated with elaborate stromal density and epithelial projections called pinapodes[15]. These receptive features for which WOI is the hallmark is related to several biomarkers such as transcription factors, cytokines, integrin, and as well as growth factors[16]. However, the prognostic value of these biomarkers through genetic profiling of endometrial cells has been subject for debate in the literature[17,18]. With pregnancy, the contact of the blastocyst and the endometrium commence the process of attachment, invasion of the trophoblast culminating the formation of the placenta[19].

HISTORICAL PERSPECTIVE

The concept of endometrial receptivity is dated back to the work of Rock and Bartlett in 1950 [20] in the course of trying to design the concept of endometrial dating. While Noyes et al. defined the secretory endometrium through histological examination of the endometrial biopsy [21]. Over time, it became apparent that the histological determination of endometrial receptivity provides irrelevant information and has little or no benefit in the clinical entity[22]. Subsequently, effort at developing biomarkers led to the evaluation of the morphological features associated with endometrial receptivity [23,24,25].

Furthermore, Hertig and Rock[26] revealed through an examination of the uterus of hysterectomized women intended to get pregnant, that the early event of implantation occurs at about day 19 of the cycle. The corroboration of the findings by other studies[27-29] led to the concept of WOI put at day[19-23] in the human menstrual cycle.

Also, the period has shown to coincide with the time serum progesterone is at its peak suggestive of the central role of progesterone in implantation[30]. The background knowledge from the in-vivo model has been explored towards the right timing of the embryo and endometrium interaction as a prelude to successful implantation in rodent[31,32] and in human.[33,34] Furthermore, the study of WOI at the molecular level has revealed its association with changes in the steroid receptors and as well as expression of other factors such as the integrin[35].

MORPHOLOGICAL CHANGES

The endometrial changes before the onset of implantation process are the accelerated cellularity of the luminal and the glandular epithelium often localized at the apex of the proposed area for the implantation[36]. The luminal change is associated with the formation of nuclear clumps epithelial plaque regulated by steroid hormones[37]. Though the

function is not well known, it is believed to have some nutritional benefit for the proposed implanted embryo through the provision of glycogen[38,39]. Also, the basal lamina become thin with the separation of the gap junction[40] to pave the way for apposition and the subsequent invasion of the trophoblast[41].

Another characteristic feature associated with the epithelial surface is the presence of Mucin 1 (MUC 1). It is a glycoprotein that forms a layer of glycocalyx upregulated during WOI. It is believed to determine the site of attachment and adhesion of the blastocyst through the process of shedding by the blastocyst secreted metalloproteinase enzyme[19,42]. The mechanism by which it promotes attachment of blastocyst during implantation has been established in mouse and non-human primates studies[43] poorly understood in the human[44,45]. Other related luminal biomarkers are MUC16 which has to be downregulated as well, L-Selectin, Heparin-binding growth factor (HBGF)[46,22].

The stromal undergoes extensive decidualization by glandular secretion and proliferation of specialized uterine Natural Killer cells and vascular permeability. The transformation creates an environment for optimal trophoblast invasion and subsequent access to maternal vascular bed[47] to promote adequate perfusion for the nascent embryo. The physiological process determines the quality of the placentation and has a lot of clinical implications[48]. With the onset of implantation, decidualization is maintained by the steroid hormones and the cellular signals[49]. Also, there is the formation of neovascularization regulated by the hormones. This is believed to be a prerequisite for the infiltrations of the immune cells and subsequent differentiation[50].

The endometrium endowed with several infiltrated immune cells. Of significance, are the uterine Natural killer cells (uNK)[51] and tends to increase during the period of implantation. During pregnancy, the uNK cells differentiate into decidual NK cells. Though the role of uNK cells is lace with controversies, a study in the mouse has shown that reduction in the decidual NK cells results in pregnancy failure[52].Other immune cells are T-lymphocytes and Macrophages.

MOLECULAR TRANSFORMATION

Cytokines and Growth Factors

The most important cytokines involved in the implantation process are Leukemia Inhibitory Factor (LIF), interleukins- 6 (IL-6) and inter leukin-11 (IL-11) with common receptor protein gp- 130 in carrying their functions[53]. The central role of LIF in implantation was first established in a knockout female mouse resulting in implantation failure due to downregulation of STAT3 signaling in the endometrial epithelium[54].Subsequently, in infertile women following demonstration of LIF and its protein expression in endometrial biopsy throughout the menstrual cycle and the association of RIF and unexplained infertility with mutation of LIF gene[55]. A Recent study has shown that LIF is involved in both adhesion and invasion of the blastocyst[30,56].

Furthermore, study with mice has shown that its receptor tends to increase expression in decidualized stromal cells close to the site of implantation and administration of antagonist resulted in the loss of pregnancy in primates and mice[57]. Expression of the IL-6 is mainly in the glandular cells[58] promoting the decidualization of the endometrium creating better access for the invasion of the trophoblast[30]. The IL-11 is expressed by all cell type in the endometrium in a cyclical manner[59] and in combination with IL-6, is involved in the decidualization of the endometrium[60]. Unlike the IL-6 whose regulation is under the influence of ovarian steroid hormones, there is no consensus about IL-11[61].

In addition to cytokines, growth factors like Transforming Growth Factors (TGF) beta promote the implantation process by its regulatory impact on the immune system[62]. Its receptors are predominantly in the glandular epithelial cells and enhance the

decidualization for adequate trophoblastic invasion. While the Tumour Necrosis Factor (TNF) alpha and Epidermal Growth Factor (EGF) has their receptors in the glandular and stromal cells with more expression in the stromal cell during early pregnancy in animals[63]. The increased level in the stromal cell is suggestive of its important role in decidualization[36].

Integrin and Ligands

Integrin and ligands (Osteopontin, Fibronectin, and Collagen) are glycoproteins that mediate the adhesion of blastocyst and endometrium. Its receptors exhibit variation in time and location during WOI. Depending on the ligand, it tends to be more expressed in the glandular epithelium at the apical region of the endometrium during the WOI and decreases in the early pregnancy[64]. However, a study with Ishikawa cells has shown that alpha4beta3 is the main receptor for osteopontin[65]. Moreover, play a central role in the process of adhesion during implantation. For example, a knockout mouse study of osteopontin (SPP1) and its receptor alpha4beta3 resulted in implantation failure[66]. Osteopontin was first recovered from the bone matrix[67] and associated with several tissues. Its expression in the secretory phase endometrium was first noted by Young et al.[14] and it coincided with the period of blastocyst attachment regulated by progesterone[68]. While progesterone regulates the expression of Osteopontin, its main receptor integrin alpha4beta3 is differentially regulated by EGF and HOXA[10] in a paracrine mode of action guaranteed by the progesterone receptor in the stromal cells[69]. The modulation also related to the association of elevated estrogen receptor alpha and downregulation of integrin during WOI. Thus, explains the correlation of elevated estrogen receptor alpha during WOI and implantation failure[9].

While the Fibronectin and its receptor alpha4beta1 are expressed mainly in the glandular cells, Osteopontin and its receptor alpha4beta3 are expressed in the glandular and stromal cells[70]. Unlike the fibronectin and Osteopontin, the Collagen and its receptor alpha1beta1 expression is lost in early pregnancy[71]. A Recent study revealed differential expression of integrin at the site of attachment of the embryo in the upper zone of the luminal epithelium about the basal distribution in the non-attached area[63]. The finding was corroborated by another study with mouse embryo and Ishikawa cells[65] suggesting the role of embryo signaling of endometrial epithelial cells in the process of implantation

Genomics and Endometrial Receptivity

An attempt at the use of the regulatory potential of molecular expression and gene targeting to evaluate abnormalities, has led to the development of genomic sequencing of the various endometrial genes involve in the process of implantation. Hence, different Omics technologies are available, and transcriptomics by microarray or RNA sequencing have been used to look at changes in a large number of transcripts in the endometrium[42,72]. The concept involves the use of Omics to analyze the genes, lipids, and proteins of the endometrium to (or "intending to") generating biomarkers that may be useful to predict endometrial receptivity.

Ponnampalam et al. first applied the technique[73] to determine the various stages of the menstrual cycle irrespective of the morphological state through the evaluation of the transcription profile of endometrial genes. As a result, verify the expression of genes during the receptive period of the endometrium.

The concept was facilitated against the backdrop of the impact of ovarian stimulation during IVF treatment on the endometrial morphology[22]. It has been shown that ovarian stimulation results in unwarranted endometrial morphological changes[74].Thus, resulting in compromised receptivity. Subsequently, the use of endometrial gene profiling became a valuable tool to determine the receptive status of the endometrium in IVF cycle prior to embryo transfer[75]. Despite the perceived benefits, the gene pool generated could not define the ideal or specific biomarker due to the heterogeneity of

the sample and variation in the cycle. So, it became unfeasible for routine clinical use[76]. Furthermore, the invasive nature of getting the endometrial sample for analysis as well as the weak correlation of gene profiling with the secreted proteins makes it less appealing. Consequently, emphasis shifted to the use of proteomics[72,77].

PROTEOMICS

Beier and Beier-Hellwig in 1998[78] were the first to demonstrate the concept of proteomics based on the significant amount of secreted fluid in the endometrial cavity in the secretory phase. Though several genes identified, the function of most the genes was not known. In one study[3,79], progesterone receptors were noted to be downregulated in the luminal epithelium during the secretory phase of the endometrium in mice and sheep. In spite, the observations, the nonuniformity of the sample constituents per sample collection renders the method unacceptable[80].

Data from animal studies have shown the importance of lipid in the endometrial receptivity[81,82]. Though, yet to be established in the human[80], a study has shown a correlation of reduced serum Lysophosphatidic acid (PLA) and Cyclooxygenase 2 (COX 2) with recurrent implantation failure in IVF Patient[83]. In the contest of lipidomics, a study has shown only high value in the PGE2 and PGF2 alpha during WOI while other parameters remained unchanged[84]. While its role in the animals seems promising, the relevance in human is still subject to debate[22].

Over the years, the concept and its related diagnostic tool of endometrial receptivity array (ERA) have demonstrated the critical role of relevant gene expression during WOI for successful implantation[85]. Despite its drawbacks about specificity[86], it has established the fact that some genes expressions are involved in the endometrial receptivity and implantation process[87]. In light of this, therefore, MicroRNAs have been considered potential regulatory elements in the concept of endometrial receptivity[4,88].

MicroRNAs

MicroRNAs are minute non-coding RNA molecules of about 18-25 nucleotides involve in the modulation of many target genes 85. The regulatory process is either by direct degradation of mRNA or inhibition of post-translation expression. Thus, influence range of biological processes[89].

The regulatory role of microRNAs in the uterine gene expression demonstrated in mouse[90]. In human, Dominguez et al.[91] showed the differential expression of 24 ovarian hormone-dependent microRNAs in the menstrual cycle. The finding corroborated by other studies[85,88] showed that twelve microRNAs are differentially expressed during the secretory phase of the endometrium and were mainly in the glandular and endothelial cells of the epithelial lining of the endometrium.

Even within the mid-secretory phase, differential expression was noted within the pre-receptive and receptive period[92].For example, microRNAs 30b and 30d are upregulated while microRNAs 494 and 932 downregulated in the receptive phase. Also, the differential expression of microRNAs 22 and 145 has been noted infertile women and those with recurrent implantation failure (RIF) due to altered endometrial microRNA profile resulting in poor endometrial receptivity[88,93]. Though, several microRNAs have been demonstrated in the process of implantation, the role of significant number is still not known[94].

Hormones (Progesterone)

The molecular interplay involved in the receptivity of the endometrium is modulated by several factors and gene expressions regulated by progesterone[85]. As a consequence of a defect in progesterone production or resistance to its receptors, implantation failure may result from altered expression of the relevant genes during WOI[69]. In light of the absolute requirement of progesterone in endometrial receptivity and maintenance of the corpus luteum of pregnancy[95], the name progesterone was borne out from the Latin word PRO and

GESTURE in 1930[96]. Studies by knockout and anti-progesterone (RU 486) Mifepristone, have demonstrated the relationship of its receptors in the genetic and phenotype expression of endometrial receptivity[97,98]. The assertion has been heightened by Labarta et al.[99], who showed the alteration of 140 endometrial genes expression with elevated progesterone level and consequent adverse effect on the endometrial receptivity in non-human primate in-vivo. Thus, give credence to the central role of progesterone in endometrial function.

Progesterone carries out its function through the receptors. The two isoforms A and B are from different slice variant in the same gene. The B isoform had an extra 164 amino acid residues at the N-terminus of the protein[95].

Despite the difference in size, a knockout study in the mouse has shown that isoform A tends to exhibit more functional attribute in the uterus[96]. Unlike in the mouse, the human isoforms, A and B function in a comparable manner, and their levels tend to vary with the menstrual cycle[100]. Furthermore, the B-isoform plays a dominant role in a situation where both isoforms determine the expression of a gene[101].

Under the influence of estrogen, at the onset of the cycle, these receptors are expressed on the epithelial lining and in the stromal cells of the endometrium[102,103]. At the secretory phase, PR-B is down-regulated while the PR-A remain only in the stromal cells for decidualization[104,105]. The expression is closely associated with the expression of Insulin Growth Factor Binding Protein-1 (IGFBP-1), a marker involved in decidualization[100].

Regulating factors of Progesterone Receptor

Various factors influence the activities of the Progesterone Receptors (PR) and its ability to manipulate the expression of the target genes. Some of these factors include the estrogen and progesterone[95]. A knockout mouse study has shown that estrogenic influence on the PR is through the presence of estrogen receptor (ERAlpha) in the stromal cells[106] and progesterone impact by negative feedback mechanism[107]. Other factors involve its combination with the immunophilins[108]. For example, immunophilins such as FKbp4 has been showed to promote the expression of a gene involves in the optimal decidualization through the suppression of estrogen-primed gene Lactoferrin (Ltf) that promote epithelial proliferation[106].So, the proliferative action of estrogen on the luminal epithelial cells needs to be suppressed during the mid-secretory phase to allow for decidualization during implantation.

In the presence of progesterone, PR disengages from the immunophilins and its activity become modulated by P160/SRC (Steroid Receptor Coactivator)[109]. SR-1 and SR-2 expressed in the epithelial and stromal cells. While, the SR-1 may be complementary. Knockout study 12 and microarray study[95,110] have shown that SR-2 plays a prominent role in progesterone mediated gene expression, and the place of SR-3 is not well established 95. Also, Kruppel-like Factor (KLf 9) 111, 112 and Bone Morphogenic Protein (BMP2) 113 are cofactors for PR towards optimal implantation.

Effectors of Progesterone Receptors

Base on the background knowledge that progesterone impact through its receptors on the endometrial receptivity by the expression of various genes in diverse signaling routes[86]. It has become imperative to gear efforts towards the determination of the exact genes influenced during the WOI. Studies[12,95] have shown that Indian hedgehog gene (Ihh) is one of such gene expressed on the epithelial lining. This mediates the expression of Patched-1 (Ptch-1) and COUP-TF11 in the stromal during implantation[114]. These mediators have been shown to be vital in decidualization by their suppression/downregulating ERalpha, preventing epithelial proliferation during WOI, culminating in successful implantation[106]. Thus, suggesting the important role of Ihh gene.

Similarly, COUP-TF11 has been noted to

promote endometrial receptivity through the expression of BMP2; a critical element expressed near the site of implantation due to its role in decidualization in murine and in the human endometrium[114]. The impact of which has been demonstrated to be through the induction of Wnt4, which promotes cell development and differentiation[115]. In addition to receptor signaling, progesterone can directly induce the expression of some genes such as Mig6 and cyclooxygenase 2 (COX-2). Mig6 produced in both epithelial as well as stromal cells can regulate the impact of estrogen and progesterone by feedback mechanism[95].

The COX-2 mediates the production of Prostaglandin (PG)86,[116] while COX-1 is more of complementary. Knockout mice have shown that COX-2 is associated with angiogenesis due to its involvement in the signaling mechanism of vascular endothelial growth factor (VEGF)[117]. Another gene under the direct influence of the progesterone is the HOX10 with the unique space and time of expression in the endometrial lumen. Knockout mice, microarray, and siRNA with human endometrial culture (HESC) studies have demonstrated the role in the attachment of blastocyst as well as decidualization[95,100] and optimal function of other progesterone modulated genes like the COX 2 and PG activities.

Furthermore, the importance of anti-estrogenic proliferation through the downregulation of ERalpha has been demonstrated in RU 486 study. The study revealed the role of Hand 2 and STAT 3 mediated progesterone activity in endometrial receptivity and enhancement of blastocyst attachment[98,100]. Thus, further emphasize the anti-proliferative role of progesterone in the luminal epithelial cells during WOI.

CONCLUSION

Embryo implantation results from a well-coordinated sequence of molecular and cellular events guaranteed by the endometrial receptivity within a time frame termed window of implantation. (WOI). Endometrial receptivity appears to pose a stumbling block in the context of reproductive process as the only limited number of pregnancy rates have resulted from various treatment modalities aim at failures of conception, despite the availability of relatively quality embryos. Studies done on endometrial receptivity have been on an animal model and cannot transpose to human because of wide species variation. The evaluation of the endometrial biomarkers in the window of implantation could serve as an adjunct to the morphological changes associated with endometrial receptivity. Therefore, research should gear towards the functional components of the endometrial receptivity. Such concept could help to develop a therapeutic intervention for recurrent implantation failure and by extension, generate a novel fertility regulation method.

REFERENCES:

1. Kaneko Y, Day ML, Murphy CR. Integrin beta3 in rat blastocysts and epithelial cells is essential for implantation in vitro: studies with Ishikawa cells and small interfering RNA transfection. Hum Reprod. 2011; 26:1665-74.
2. Kang Y-J, Forbes K, Carver J, Aplin JD. The role of the osteopontin–integrin αvβ3 interaction at implantation: functional analysis using three different in vitro models. Human Reproduction. 2014:det433.
3. Díaz-Gimeno P, Horcajadas JA, Martínez-Conejero JA, Esteban FJ, Alamá P, Pellicer A, et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. Fertility and sterility. 2011; 95:50-60. e15.
4. Garrido-Gómez T, Ruiz-Alonso M, Blesa D, Diaz-Gimeno P, Vilella F, Simón C. Profiling the gene signature of endometrial receptivity: clinical results. Fertility and sterility. 2013; 99:1078-85.
5. Foulk RA, Zdravkovic T, Genbacev O, Prakobphol A. Expression of L-selectin ligand MECA-79 as a predictive marker

of human uterine receptivity. Journal of assisted reproduction and genetics. 2007; 24:316-21.
6. Hawkins SM, Matzuk MM. The menstrual cycle: basic biology. Annals of the New York Academy of Sciences. 2008; 1135:10-8.
7. Trukhacheva E, Lin Z, Reierstad S, Cheng YH, Milad M, Bulun SE. Estrogen receptor (ER) beta regulates ERalpha expression in stromal cells derived from ovarian endometriosis. The Journal of clinical endocrinology and metabolism. 2009; 94:615-22.
8. Bulun SE, Monsavais D, Pavone ME, Dyson M, Xue Q, Attar E, et al. Role of estrogen receptor-beta in endometriosis. Seminars in reproductive medicine. 2012; 30:39-45.
9. Lessey BA, Palomino WA, Apparao KB, Young SL, Lininger RA. Estrogen receptor-alpha (ER-alpha) and defects in uterine receptivity in women. Reproductive biology and endocrinology : RB&E. 2006; 4 Suppl 1:S9.
10. Lessey BA. Assessment of endometrial receptivity. Fertility and sterility. 2011; 96:522-9.
11. Saha P, Saraswat G, Chakraborty P, Banerjee S, Pal BC, Kabir SN. Puerarin, a selective oestrogen receptor modulator, disrupts pregnancy in rats at pre-implantation stage. Reproduction (Cambridge, England). 2012; 144:633-45.
12. Lee KY, Jeong JW, Tsai SY, Lydon JP, DeMayo FJ. Mouse models of implantation. Trends in endocrinology and metabolism: TEM. 2007; 18:234-9.
13. Jabbour HN, Kelly RW, Fraser HM, Critchley HO. Endocrine regulation of menstruation. Endocrine reviews. 2006; 27:17-46.
14. Young SL. Oestrogen and progesterone action on endometrium: a translational approach to understanding endometrial receptivity. Reproductive biomedicine online. 2013; 27:497-505.
15. Tu Z, Ran H, Zhang S, Xia G, Wang B, Wang H. Molecular determinants of uterine receptivity. Int J Dev Biol. 2014; 58:147-54.
16. Zhang S, Kong S, Lu J, Wang Q, Chen Y, Wang W, et al. Deciphering the molecular basis of uterine receptivity. Molecular reproduction and development. 2013; 80:8-21.
17. Cha J, Vilella F, Dey SK, Simon C. Molecular interplay in successful implantation. Ten Critical Topics in Reproductive Medicine Washington, DC: Science/AAA. 2013:44-8.
18. Hiraoka T, Saito-Fujita T, Hirota Y. How does Progesterone Support Embryo Implantation? Journal of Mammalian Ova Research. 2015; 32:87-94.
19. Dekel N, Gnainsky Y, Granot I, Racicot K, Mor G. The role of inflammation for a successful implantation. American journal of reproductive immunology (New York, NY : 1989). 2014; 72:141-7.
20. Rock J, Bartlett MK. Biopsy studies of human endometrium: criteria of dating and information about amenorrhea, menorrhagia, and time of ovulation. Journal of the American Medical Association. 1937; 108:2022-8.
21. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. American journal of obstetrics and gynecology. 1975; 122:262-3.
22. von Grothusen C, Lalitkumar S, Boggavarapu NR, Gemzell-Danielsson K, Lalitkumar PG. Recent advances in understanding endometrial receptivity: molecular basis and clinical applications. American journal of reproductive immunology (New York, NY : 1989). 2014; 72:148-57.
23. Haouzi D, Dechaud H, Assou S, De Vos J, Hamamah S. Insights into human endometrial receptivity from transcriptomic and proteomic data. Reproductive biomedicine online. 2012; 24:23-34.
24. Fritz R, Jain C, Armant DR. Cell signaling in trophoblast-uterine communication. Int J Dev Biol. 2014; 58:261-71.
25. Pitman H, Innes BA, Robson SC, Bulmer JN, Lash GE. Altered expression of

interleukin-6, interleukin-8 and their receptors in decidua of women with sporadic miscarriage. Human Reproduction. 2013; 28:2075-86.
26. Hertig AT, Rock J, Adams EC. A description of 34 human ova within the first 17 days of development. The American journal of anatomy. 1956; 98:435-93.
27. Navot D, Anderson TL, Droesch K, Scott RT, Kreiner D, Rosenwaks Z. Hormonal manipulation of endometrial maturation. The Journal of clinical endocrinology and metabolism. 1989; 68:801-7.
28. Khonelidze NL, Tsagareishvili GG, Koiava MA, Osidze KR. [The role of ultrasound scanning of endometrium in the superovulation stimulation program during in vitro fertilization and embryo transfer]. Georgian medical news. 2005:20-2.
29. Casper RF. It's time to pay attention to the endometrium. Fertility and sterility. 2011; 96:519-21.
30. Rashid NA, Lalitkumar S, Lalitkumar PG, Gemzell-Danielsson K. Endometrial receptivity and human embryo implantation. American Journal of Reproductive Immunology. 2011; 66:23-30.
31. Pope WF. Uterine asynchrony: a cause of embryonic loss. Biology of reproduction. 1988; 39:999-1003.
32. Wiltbank MC, Baez GM, Garcia-Guerra A, Toledo MZ, Monteiro PL, Melo LF, et al. Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows. Theriogenology. 2016; 86:239-53.
33. Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. The New England journal of medicine. 1999; 340:1796-9.
34. Lucas ES, Dyer NP, Murakami K, Hou Lee Y, Chan YW, Grimaldi G, et al. Loss of Endometrial Plasticity in Recurrent Pregnancy Loss. Stem cells (Dayton, Ohio). 2016; 34:346-56.
35. Pan X, Wang X, Wang Z, Wang X, Dou Z, Li Z. Bisphenol a influences blastocyst implantation via regulating integrin beta3 and trophinin expression levels. International journal of clinical and experimental medicine. 2015; 8:20035-45.
36. Modi DN, Godbole G, Suman P, Gupta SK. Endometrial biology during trophoblast invasion. Front Biosci (Schol Ed). 2012; 4:1151-71.
37. Buck VU, Gellersen B, Leube RE, Classen-Linke I. Interaction of human trophoblast cells with gland-like endometrial spheroids: a model system for trophoblast invasion. Human reproduction (Oxford, England). 2015; 30:906-16.
38. Enders AC, Welsh AO, Schlafke S. Implantation in the rhesus monkey: Endometrial responses. The American journal of anatomy. 1985; 173:147-69.
39. Xiong T, Zhao Y, Hu D, Meng J, Wang R, Yang X, et al. Administration of calcitonin promotes blastocyst implantation in mice by up-regulating integrin beta3 expression in endometrial epithelial cells. Human reproduction (Oxford, England). 2012; 27:3540-51.
40. Weimar CH, Macklon NS, Post Uiterweer ED, Brosens JJ, Gellersen B. The motile and invasive capacity of human endometrial stromal cells: implications for normal and impaired reproductive function. Human reproduction update. 2013; 19:542-57.
41. Rosario GX, Hondo E, Jeong JW, Mutalif R, Ye X, Yee LX, et al. The LIF-mediated molecular signature regulating murine embryo implantation. Biology of reproduction. 2014; 91:66.
42. Singh H, Nardo L, Kimber SJ, Aplin JD. Early stages of implantation as revealed by an in vitro model. Reproduction (Cambridge, England). 2010; 139:905-14.
43. Bastu E, Mutlu MF, Yasa C, Dural O, Nehir Aytan A, Celik C, et al. Role of Mucin 1 and Glycodelin A in recurrent implantation failure. Fertil Steril. 2015; 103:1059-64.e2.
44. Aplin JD. Embryo implantation: the

molecular mechanism remains elusive. Reproductive biomedicine online. 2006; 13:833-9.
45. Raheem KA, Marei WF, Campbell BK, Fouladi-Nashta AA. In vivo and in vitro studies of MUC1 regulation in sheep endometrium. Theriogenology. 2016; 85:1635-43.
46. Lai TH, Zhao Y, Shih Ie M, Ho CL, Bankowski B, Vlahos N. Expression of L-selectin ligands in human endometrium during the implantation window after controlled ovarian stimulation for oocyte donation. Fertil Steril. 2006; 85:761-3.
47. Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. Endocr Rev. 2014; 35:851-905.
48. Saleh L, Otti GR, Fiala C, Pollheimer J, Knofler M. Evaluation of human first trimester decidual and telomerase-transformed endometrial stromal cells as model systems of in vitro decidualization. Reproductive biology and endocrinology : RB&E. 2011; 9:155.
49. Wei X, Xiaoling Z, Kai M, Rui W, Jing X, Min G, et al. Characterization and comparative analyses of transcriptomes for in vivo and in vitro produced peri-implantation conceptuses and endometria from sheep. The Journal of reproduction and development. 2016.
50. Pence JC, Clancy KB, Harley BA. The induction of pro-angiogenic processes within a collagen scaffold via exogenous estradiol and endometrial epithelial cells. Biotechnology and bioengineering. 2015; 112:2185-94.
51. Lash GE, Bulmer JN, Li TC, Innes BA, Mariee N, Patel G, et al. Standardisation of uterine natural killer (uNK) cell measurements in the endometrium of women with recurrent reproductive failure. Journal of reproductive immunology. 2016; 116:50-9.
52. Gong X, Liu Y, Chen Z, Xu C, Lu Q, Jin Z. Insights into the paracrine effects of uterine natural killer cells. Molecular medicine reports. 2014; 10:2851-60.
53. Mathew DJ, Lucy MC, R DG. Interleukins, interferons, and establishment of pregnancy in pigs. Reproduction (Cambridge, England). 2016; 151:R111-22.
54. Kubota Y, Hirashima M, Kishi K, Stewart CL, Suda T. Leukemia inhibitory factor regulates microvessel density by modulating oxygen-dependent VEGF expression in mice. The Journal of clinical investigation. 2008; 118:2393-403.
55. Tawfeek MA, Eid MA, Hasan AM, Mostafa M, El-Serogy HA. Assessment of leukemia inhibitory factor and glycoprotein 130 expression in endometrium and uterine flushing: a possible diagnostic tool for impaired fertility. BMC women's health. 2012; 12:10.
56. Suman P, Malhotra SS, Gupta SK. LIF-STAT signaling and trophoblast biology. Jak-stat. 2013; 2:e25155.
57. Terakawa J, Wakitani S, Sugiyama M, Inoue N, Ohmori Y, Kiso Y, et al. Embryo implantation is blocked by intraperitoneal injection with anti-LIF antibody in mice. The Journal of reproduction and development. 2011; 57:700-7.
58. Cronin JG, Kanamarlapudi V, Thornton CA, Sheldon IM. Signal transducer and activator of transcription-3 licenses Toll-like receptor 4-dependent interleukin (IL)-6 and IL-8 production via IL-6 receptor-positive feedback in endometrial cells. Mucosal immunology. 2016.
59. Menkhorst E, Salamonsen LA, Zhang J, Harrison CA, Gu J, Dimitriadis E. Interleukin 11 and activin A synergise to regulate progesterone-induced but not cAMP-induced decidualization. Journal of reproductive immunology. 2010; 84:124-32.
60. Winship AL, Koga K, Menkhorst E, Van Sinderen M, Rainczuk K, Nagai M, et al. Interleukin-11 alters placentation and causes preeclampsia features in mice. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112:15928-33.
61. Singh M, Chaudhry P, Asselin E. Bridging endometrial receptivity and implantation:

network of hormones, cytokines, and growth factors. The Journal of endocrinology. 2011; 210:5-14.
62. Omwandho CO, Konrad L, Halis G, Oehmke F, Tinneberg HR. Role of TGF-betas in normal human endometrium and endometriosis. Human reproduction (Oxford, England). 2010; 25:101-9.
63. Nimbkar-Joshi S, Katkam RR, Kakar R, Singh P, Chaudhari UK, Manjramkar DD, et al. Proliferation and decidualization of endometrial stromal cells during embryo-attachment stage in bonnet monkeys (Macaca radiata). Cell and tissue research. 2015; 361:605-17.
64. Davidson LM, Coward K. Molecular mechanisms of membrane interaction at implantation. Birth defects research Part C, Embryo today : reviews. 2016; 108:19-32.
65. Kang YJ, Forbes K, Carver J, Aplin JD. The role of the osteopontin-integrin alphavbeta3 interaction at implantation: functional analysis using three different in vitro models. Human reproduction (Oxford, England). 2014; 29:739-49.
66. Liu N, Zhou C, Chen Y, Zhao J. The involvement of osteopontin and beta3 integrin in implantation and endometrial receptivity in an early mouse pregnancy model. European journal of obstetrics, gynecology, and reproductive biology. 2013; 170:171-6.
67. Apparao KB, Murray MJ, Fritz MA, Meyer WR, Chambers AF, Truong PR, et al. Osteopontin and its receptor alphavbeta(3) integrin are coexpressed in the human endometrium during the menstrual cycle but regulated differentially. The Journal of clinical endocrinology and metabolism. 2001; 86:4991-5000.
68. Johnson GA, Burghardt RC, Bazer FW. Osteopontin: a leading candidate adhesion molecule for implantation in pigs and sheep. Journal of animal science and biotechnology. 2014; 5:56.
69. Young SL, Lessey BA. Progesterone function in human endometrium: clinical perspectives. Seminars in reproductive medicine. 2010; 28:5-16.
70. Mangale SS, Modi DN, Reddy KV. Identification of genes regulated by an interaction between alphavbeta3 integrin and vitronectin in murine decidua. Reproduction, fertility, and development. 2008; 20:311-9.
71. Milner R. Microglial expression of alphavbeta3 and alphavbeta5 integrins is regulated by cytokines and the extracellular matrix: beta5 integrin null microglia show no defects in adhesion or MMP-9 expression on vitronectin. Glia. 2009; 57:714-23.
72. Demiral İ, Doğan M, Bastu E, Buyru F. Genomic, proteomic and lipidomic evaluation of endometrial receptivity. nutrition. 14:18-21.
73. Ponnampalam AP, Weston GC, Susil B, Rogers PA. Molecular profiling of human endometrium during the menstrual cycle. The Australian & New Zealand journal of obstetrics & gynaecology. 2006; 46:154-8.
74. Nastri CO, Polanski LT, Raine-Fenning N, Martins WP. Endometrial scratching for women with repeated implantation failure. Human reproduction (Oxford, England). 2014; 29:2855-6.
75. Shohayeb A, El-Khayat W. Does a single endometrial biopsy regimen (S-EBR) improve ICSI outcome in patients with repeated implantation failure? A randomised controlled trial. European journal of obstetrics, gynecology, and reproductive biology. 2012; 164:176-9.
76. Ruiz-Alonso M, Blesa D, Diaz-Gimeno P, Gomez E, Fernandez-Sanchez M, Carranza F, et al. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil Steril. 2013; 100:818-24.
77. Diaz-Gimeno P, Ruiz-Alonso M, Blesa D, Simon C. Transcriptomics of the human endometrium. Int J Dev Biol. 2014; 58:127-37.
78. Beier HM, Beier-Hellwig K. Molecular and cellular aspects of endometrial

receptivity. Human reproduction update. 1998; 4:448-58.
79. Forde N, Simintiras CA, Sturmey R, Mamo S, Kelly AK, Spencer TE, et al. Amino acids in the uterine luminal fluid reflects the temporal changes in transporter expression in the endometrium and conceptus during early pregnancy in cattle. PloS one. 2014; 9:e100010.
80. Berlanga O, Bradshaw H, Vilella-Mitjana F, Garrido-Gomez T, Simon C. How endometrial secretomics can help in predicting implantation. Placenta. 2011; 32:S271-S5.
81. Vilella F, Ramirez LB, Simón C. Lipidomics as an emerging tool to predict endometrial receptivity. Fertility and sterility. 2013; 99:1100-6.
82. Forde N, McGettigan PA, Mehta JP, O'Hara L, Mamo S, Bazer FW, et al. Proteomic analysis of uterine fluid during the pre-implantation period of pregnancy in cattle. Reproduction (Cambridge, England). 2014; 147:575-87.
83. Achache H, Tsafrir A, Prus D, Reich R, Revel A. Defective endometrial prostaglandin synthesis identified in patients with repeated implantation failure undergoing in vitro fertilization. Fertil Steril. 2010; 94:1271-8.
84. Sordelli MS, Beltrame JS, Cella M, Gervasi MG, Martinez SP, Burdet J, et al. Interaction between lysophosphatidic acid, prostaglandins and the endocannabinoid system during the window of implantation in the rat uterus. PloS one. 2012; 7:e46059.
85. Galliano D, Pellicer A. MicroRNA and implantation. Fertil Steril. 2014; 101:1531-44.
86. Li J, Liang X, Chen Z. Improving the embryo implantation via novel molecular targets. Current drug targets. 2013; 14:864-71.
87. Altmae S, Esteban FJ, Stavreus-Evers A, Simon C, Giudice L, Lessey BA, et al. Guidelines for the design, analysis and interpretation of 'omics' data: focus on human endometrium. Human reproduction update. 2014; 20:12-28.
88. Liu W, Niu Z, Li Q, Pang RT, Chiu PC, Yeung WS. MicroRNA and Embryo Implantation. American journal of reproductive immunology (New York, NY : 1989). 2016; 75:263-71.
89. Imbar T, Galliano D, Pellicer A, Laufer N. Introduction: MicroRNAs in human reproduction: small molecules with crucial regulatory roles. Fertil Steril. 2014; 101:1514-5.
90. Hu SJ, Ren G, Liu JL, Zhao ZA, Yu YS, Su RW, et al. MicroRNA expression and regulation in mouse uterus during embryo implantation. The Journal of biological chemistry. 2008; 283:23473-84.
91. Dominguez F, Moreno-Moya JM, Lozoya T, Romero A, Martinez S, Monterde M, et al. Embryonic miRNA profiles of normal and ectopic pregnancies. PloS one. 2014; 9:e102185.
92. Sha AG, Liu JL, Jiang XM, Ren JZ, Ma CH, Lei W, et al. Genome-wide identification of micro-ribonucleic acids associated with human endometrial receptivity in natural and stimulated cycles by deep sequencing. Fertil Steril. 2011; 96:150-5.e5.
93. Kang YJ, Lees M, Matthews LC, Kimber SJ, Forbes K, Aplin JD. MiR-145 suppresses embryo-epithelial juxtacrine communication at implantation by modulating maternal IGF1R. Journal of cell science. 2015; 128:804-14.
94. Buckberry S, Bianco-Miotto T, Roberts CT. Imprinted and X-linked non-coding RNAs as potential regulators of human placental function. Epigenetics. 2014; 9:81-9.
95. Large MJ, DeMayo FJ. The regulation of embryo implantation and endometrial decidualization by progesterone receptor signaling. Molecular and cellular endocrinology. 2012; 358:155-65.
96. Wetendorf M, DeMayo FJ. The progesterone receptor regulates implantation, decidualization, and glandular development via a complex paracrine signaling network. Molecular and cellular endocrinology. 2012;

357:108-18.
97. Franco HL, Jeong JW, Tsai SY, Lydon JP, DeMayo FJ. In vivo analysis of progesterone receptor action in the uterus during embryo implantation. Seminars in cell & developmental biology. 2008; 19:178-86.
98. Mestre-Citrinovitz AC, Kleff V, Vallejo G, Winterhager E, Saragueta P. A Suppressive Antagonism Evidences Progesterone and Estrogen Receptor Pathway Interaction with Concomitant Regulation of Hand2, Bmp2 and ERK during Early Decidualization. PloS one. 2015; 10:e0124756.
99. Labarta E, Martinez-Conejero JA, Alama P, Horcajadas JA, Pellicer A, Simon C, et al. Endometrial receptivity is affected in women with high circulating progesterone levels at the end of the follicular phase: a functional genomics analysis. Human reproduction (Oxford, England). 2011; 26:1813-25.
100. Kaya HS, Hantak AM, Stubbs LJ, Taylor RN, Bagchi IC, Bagchi MK. Roles of progesterone receptor A and B isoforms during human endometrial decidualization. Molecular endocrinology (Baltimore, Md). 2015; 29:882-95.
101. Mazur EC, Vasquez YM, Li X, Kommagani R, Jiang L, Chen R, et al. Progesterone receptor transcriptome and cistrome in decidualized human endometrial stromal cells. Endocrinology. 2015; 156:2239-53.
102. Spencer TE, Forde N, Lonergan P. The role of progesterone and conceptus-derived factors in uterine biology during early pregnancy in ruminants. Journal of dairy science. 2015.
103. Taraborrelli S. Physiology, production and action of progesterone. Acta obstetricia et gynecologica Scandinavica. 2015; 94 Suppl 161:8-16.
104. Gao J, Mazella J, Tang M, Tseng L. Ligand-activated progesterone receptor isoform hPR-A is a stronger transactivator than hPR-B for the expression of IGFBP-1 (insulin-like growth factor binding protein-1) in human endometrial stromal cells. Molecular endocrinology (Baltimore, Md). 2000; 14:1954-61.
105. Petousis S, Prapas Y, Margioula-Siarkou C, Milias S, Ravanos K, Kalogiannidis I, et al. Expression of progesterone receptors is significantly impaired in the endometrium of infertile women during the implantation window: a prospective observational study. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2016:1-8.
106. Lee D-K, Kurihara I, Jeong J-W, Lydon JP, DeMayo FJ, Tsai M-J, et al. Suppression of ERα activity by COUP-TFII is essential for successful implantation and decidualization. Molecular Endocrinology. 2010; 24:930-40.
107. Filant J, Zhou H, Spencer TE. Progesterone inhibits uterine gland development in the neonatal mouse uterus. Biology of reproduction. 2012; 86:146.
108. Tranguch S, Smith DF, Dey SK. Progesterone receptor requires a co-chaperone for signalling in uterine biology and implantation. Reproductive biomedicine online. 2007; 14 Spec No 1:39-48.
109. Jeong W, Jung S, Bazer FW, Song G, Kim J. Epidermal growth factor: Porcine uterine luminal epithelial cell migratory signal during the peri-implantation period of pregnancy. Molecular and cellular endocrinology. 2016; 420:66-74.
110. Mukherjee A, Soyal SM, Fernandez-Valdivia R, Gehin M, Chambon P, Demayo FJ, et al. Steroid receptor coactivator 2 is critical for progesterone-dependent uterine function and mammary morphogenesis in the mouse. Molecular and cellular biology. 2006; 26:6571-83.
111. Zhang XL, Zhang D, Michel FJ, Blum JL, Simmen FA, Simmen RC. Selective interactions of Kruppel-like factor 9/basic

transcription element-binding protein with progesterone receptor isoforms A and B determine transcriptional activity of progesterone-responsive genes in endometrial epithelial cells. The Journal of biological chemistry. 2003; 278:21474-82.

112.Pabona JM, Zeng Z, Simmen FA, Simmen RC. Functional differentiation of uterine stromal cells involves cross-regulation between bone morphogenetic protein 2 and Kruppel-like factor (KLF) family members KLF9 and KLF13. Endocrinology. 2010; 151:3396-406.

113.Nagashima T, Li Q, Clementi C, Lydon JP, DeMayo FJ, Matzuk MM. BMPR2 is required for postimplantation uterine function and pregnancy maintenance. The Journal of clinical investigation. 2013; 123:2539-50.

114.Kurihara I, Lee D-K, Petit FG, Jeong J, Lee K, Lydon JP, et al. COUP-TFII mediates progesterone regulation of uterine implantation by controlling ER activity. PLoS Genet. 2007; 3:e102.

115.Franco HL, Dai D, Lee KY, Rubel CA, Roop D, Boerboom D, et al. WNT4 is a key regulator of normal postnatal uterine development and progesterone signaling during embryo implantation and decidualization in the mouse. The FASEB Journal. 2011; 25:1176-87.

116.Sun T, Li SJ, Diao HL, Teng CB, Wang HB, Yang ZM. Cyclooxygenases and prostaglandin E synthases in the endometrium of the rhesus monkey during the menstrual cycle. Reproduction (Cambridge, England). 2004; 127:465-73.

117.Douglas NC, Tang H, Gomez R, Pytowski B, Hicklin DJ, Sauer CM, et al. Vascular endothelial growth factor receptor 2 (VEGFR-2) functions to promote uterine decidual angiogenesis during early pregnancy in the mouse. Endocrinology. 2009; 150:3845-54.