



Review

Molecular aspects of implantation failure[☆]Y.E.M. Koot^{a,*}, G. Teklenburg^a, M.S. Salker^b, J.J. Brosens^c, N.S. Macklon^{a,d}^a Department of Reproductive Medicine and Gynaecology, University Medical Centre Utrecht, F05.126, PO Box 85500, 3508 GA, Utrecht, The Netherlands^b Institute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Hospital, London W12 0NN, UK^c Division of Reproductive Health, Warwick Medical School, University of Warwick, Coventry CV4 7AL, UK^d Department of Obstetrics and Gynaecology, Division of Human Development and Health, University of Southampton, Princess Anne Hospital, Coxford Road, Southampton, SO16 5YA, UK

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ABSTRACT

Despite expanding global experience with advanced reproductive technologies, the majority of IVF attempts do not result in a successful pregnancy, foremost as a result of implantation failure. The process of embryo implantation, a remarkably dynamic and precisely controlled molecular and cellular event, appears inefficient in humans and is poorly understood. However, insights gained from clinical implantation failure, early pregnancy loss, and emerging technologies that enable molecular interrogation of endometrial–embryo interactions are unravelling this major limiting step in human reproduction. We review current molecular concepts thought to underlie implantation failure, consider the contribution of embryonic and endometrial factors, and discuss the clinical value of putative markers of impaired endometrial receptivity. Finally we highlight the nature of the dialogue between the maternal endometrium and the implanting embryo and discuss the concept of natural embryo selection. This article is part of a Special Issue entitled: Molecular Genetics of Human Reproductive Failure.

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1. Introduction

Pregnancy is a unique biological phenomenon. To occur, a competent embryo must attach to a receptive endometrial lining and then invade the underlying decidualizing stroma. Although our knowledge of the molecular mechanisms that govern these early embryo–maternal interactions has increased substantially in recent years, implantation remains the least understood key rate-limiting step in assisted reproductive technology (ART). While normal implantation events in human beings cannot be studied directly, analysis of the factors that contribute to IVF treatment failure do provide insight into the critical steps that determine reproductive success. The aim of this review is to outline our current understanding of embryo implantation failure based on clinical evidence and emerging concepts.

2. Implantation and implantation failure

Implantation is considered to occur when a blastocyst breaches the luminal endometrial epithelium. However, determining precisely when this occurs in the human being is complicated. The only established clinical marker of implantation is human chorionic gonadotrophin (hCG). This glycoprotein hormone, produced by the cytotrophoblast cells of the human blastocyst, is first detectable in

urine and blood a few days after implantation and then rises exponentially [1–3]. In ART, implantation is defined as a quantitative rise in hCG level above a threshold level at some point after embryo transfer [4]. The most accurate way to determine the prevalence of implantation events after IVF would be to measure hCG levels in a daily serum sample after embryo transfer. However, this approach is cumbersome and difficult to perform in large numbers of patients. Serial urine sampling offers a less invasive approach and more practical approach to study larger populations [5–7]. It has also been shown to provide data of similar validity as analysis of serum samples [8,9].

The earlier hCG is measured, the more implantation events will be captured, including transient events. Consequently, more cases of early post-implantation pregnancy loss will also be detected [5,6,10]. Thus, the frequency of implantation and implantation failure depends on detection methods and clinical definition.

The clinical definition of ‘recurrent’ implantation failure (RIF) is equally challenging and arbitrary. The 2005 ESHRE Preimplantation Genetic Diagnosis (PGD) consortium defined the criteria for RIF as the absence of implantation after ≥ 3 embryo transfers with high-quality embryos or after replacement of a total of 10 or more embryos in multiple transfers, with the exact numbers to be determined by each centre [11].

If RIF is a disorder affecting specific patients, implantation rates should correlate inversely with the number of unsuccessful IVF attempts, reflecting the increasing proportion of true RIF patients. Indeed, studies in oocyte donation programmes show that the pregnancy rate is comparable, approximately 36%, during the first 4 treatment cycles [12]. However, in IVF programmes, pregnancy rates already decline in the

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second cycle and this trend continues in subsequent cycles [13,14]. Therefore, a definition of RIF as three failed IVF cycles is inevitably arbitrary. Further, the definition should include the number of good-quality embryos transferred without achieving a pregnancy. This inclusion criterion is increasingly applied in recent studies [15–18].

To determine the rate of implantation failure in a given patient cohort, it is necessary to first establish the frequency of implantation. This represents the sum of the number of clinical pregnancies and pre-clinical pregnancy losses per embryo transfer [10]. Based on the most recent international datasets from Europe, America and Australia/New Zealand, the clinical pregnancy per transfer is 30–35% [19–21]. This is based on a mean transfer of 2 embryos (range: 1–4). These clinical pregnancy rates are comparable with the 32% reported by Boomsma et al. [10]. In this study, the total implantation rate was 51%, which included a pre-clinical pregnancy loss rate of 19%. Thus, while half of all transferred embryos resulted in a detectable implantation event, half of these were subsequently lost (Fig. 1) [10]. In the absence of consensus criteria, the incidence of RIF is difficult to determine.

In our unit in Utrecht, 10% of patients fail to achieve a clinical pregnancy after 3 IVF/ICSI attempts. Excluding patients over 38 years of age and those with poor ovarian response, the incidence of RIF fell to 4%. If defined by failure to achieve a clinical pregnancy after cumulative transfer of 10 or more good quality embryos, the incidence of RIF would be even lower.

3. Mother or embryo?

Whether the primary cause of implantation failure lies with the mother or the embryo is a longstanding and as yet unresolved question. This reflects the difficulties of interrogating the early stages of nidation in humans. Because of this, our understanding of the mechanisms that control early implantation events comes primarily from animal models, particularly from gene deletion studies in mice [22,23]. While these models provide important clues, the degree to which these data can be extrapolated to human implantation events is limited. For instance, endometrial receptivity in the mouse uterus is associated with a decrease in mucin expression [24] whereas the opposite reportedly occurs during the window of implantation in humans [25]. Another case in point is leukaemia inhibitory factor (LIF), which is indispensable for implantation in the mouse [23]. Whether or not this is also true in humans remains controversial [26,27].

In addition to animal models, several *in vitro* models have been established to study embryo–endometrial interactions [28–30]. These models have yielded some unexpected observations regarding the nature of endometrial–embryonic interactions that seems specific to the human situation. For example, emerging evidence suggests that decidualizing stromal cells are adapted to selectively recognize developmentally impaired human embryos. Furthermore, defects in this process of maternal biosensing of embryo quality are thought to facilitate implantation of poor quality embryos and compromise the development of normal embryos, thus causing recurrent miscarriages [31,32,17,33].

4. The embryo in implantation failure

Poor embryo quality is considered to be the major cause of implantation failure [34], and by and large reflects the high incidence of chromosomal abnormalities reported for human embryos [35–37]. The frequency of embryonic genetic abnormality increases with age [38,39] but also appears higher among infertile couples than in the general population [35]. These abnormalities may arise from an error during meiosis, resulting in a uniform abnormality present in all cells, or from segregation errors occurring during the first mitotic divisions, resulting in chromosomal mosaicism. Mosaicism has been reported to affect up to 91% of human embryos in the early stages of pre-implantation development

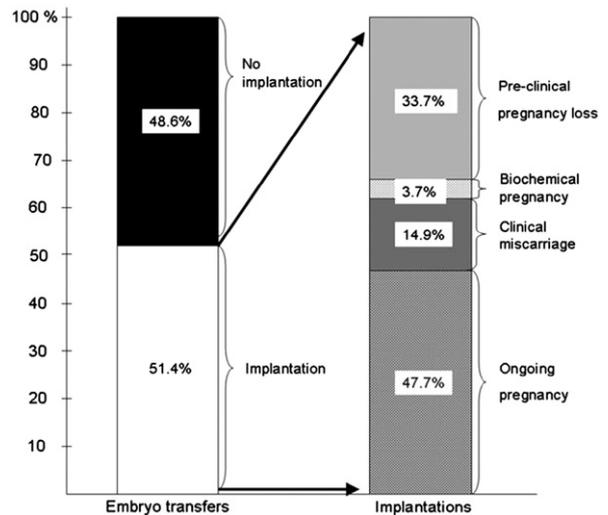


Fig. 1. Treatment outcome after embryo transfer (n = 179) based on hCG measurement in serial daily urine samples collected 9–19 days after oocyte retrieval. Adapted from Boomsma et al. [10], with permission.

[40,41,37]. However, the incidence of mosaicism is significantly lower when the embryo reaches the blastocyst stage [42], which could be explained by the developmental arrest of a significant proportion of mosaic day 4 embryos and/or reduced proliferation or selective apoptosis of aneuploid blastomeres within a mosaic embryo [42]. At the blastocyst stage, a majority of embryos are thought to be uniformly euploid [43].

Studies on couples experiencing RIF show that the proportion of chaotic mosaic embryos may be considerably higher than in unselected IVF patients [41,44]. Theoretically then, pre-implantation genetic screening (PGS) could possibly help to choose the right embryo to transfer and improve outcomes in these patients. However, a randomized controlled trial including 139 RIF patients showed no increase in implantation rate after PGS using FISH technique [15]. Further, studies using the genome-wide array comparative genome hybridization (CGH) screening have reported exceptional high rates of mosaicism in human embryos [37], raising doubts as to whether preimplantation genetic screening (PGS), with either CGH or single nucleotide polymorphism (SNP) arrays, will improve live birth rates. Additional randomised controlled trials (RCTs) are needed before these new technologies can be introduced in clinical management [45].

Metabolic profiling has been employed to determine amino acid turnover in embryo culture medium [46,47]. A low metabolic rate, characteristic of so-called 'silent embryos', is associated with developmental competence, whereas poor quality embryos operate at higher metabolite or nutrient turnover rates ('noisy embryos') [48,49]. Proteomic studies indicated that the embryonic secretome may differ between those that implant and those that fail, although prospective validation studies are as yet lacking [50,51].

In some cases RIF may be linked to an inability of the embryo to hatch out of its zona pellucida [52]. The zona pellucida is a glycoprotein layer, which after fertilization, compresses and shapes the embryo. The zona facilitates the active transport of the embryo through the Fallopian tubes and protects it from micro-organisms and immune cells [53]. At the blastocyst stage, the embryo needs to break out of the zona to enable invasion of the luminal endometrial epithelium [54]. Failure of this process could be caused by zona hardening arising from IVF culture conditions [55] or cryopreservation [56]. Further, advanced endometrial development caused by ovarian stimulation [57] combined with delayed development of embryos *in vitro*

[58] could cause a synchronization problem, which requires the embryo to hatch out of the zona prematurely.

Assisted hatching is the artificial rupture of the zona pellucida, which might assist implantation of fresh or cryopreserved IVF embryos [52]. A systematic review of 28 studies showed that assisted hatching significantly increases the clinical pregnancy rate of frozen-thawed embryos in patients with repeated treatment failure. However, assisted hatching also increases multiple pregnancy rates, including monozygotic twinning [59]. A Cochrane review in 2009 showed similar results in the RIF subgroup and concluded that while the clinical pregnancy rate may improve, there is insufficient data on live birth rates and a concerning increase in multiple pregnancies [60].

5. Endometrial factors in implantation failure

The endometrium is a multi-layered, dynamic mucosa that overlies the myometrium of the uterus. It comprises a variety of cells, including luminal and glandular epithelial cells, stromal fibroblasts, and vascular and immune cells. During a menstrual cycle, dramatic changes occur in both the phenotype and abundance of many of these cells, especially in the superficial endometrial layer [61]. Endometrial growth is dependent on oestrogen stimulation whereas the postovulatory rise in progesterone levels triggers a coordinated programme of differentiation, characterized by proliferative arrest and secretory transformation of the epithelial cells, transient oedema, influx of uterine natural cells (uNK), vascular remodelling, and differentiation of stromal fibroblasts into specialized decidual cells [61,62]. A functional consequence of this coordinated remodelling of the endometrium is that it transiently becomes receptive to embryo implantation. This phenomenon is referred to as the 'window of implantation' (WOI) [63]. It starts approximately 6 days after ovulation and thought to last for ~4 days [64]. The end of the implantation window, the refractory phase, coincides with the morphological differentiation of endometrial fibroblasts into secretory, epitheloid decidual cells [65]. The decidual process is indispensable for pregnancy in all species with an invasive placenta, as it establishes maternal immunologic tolerance to foetal antigens, ensures tissue integrity and haemostasis during the process of trophoblast invasion, and, importantly, protects the conceptus from environmental insults [65]. In the vast majority of species, decidualization of the endometrial stromal compartment is triggered by signals from the implanting embryo. However, this is not the case in human beings or Old World Monkeys [66,67]. In these species, decidualization occurs in each cycle and, in the absence of pregnancy, this process is responsible for menstrual shedding of the superficial endometrium [61].

5.1. Morphological markers of endometrial receptivity

A restricted 'window of implantation' is thought to coordinate embryonic and endometrial development, thus minimizing the risk of late implantation of non-viable embryos. However, failure of the endometrium to become receptive is widely thought to be major cause of implantation failure. For over half a century, histological dating was the gold standard to detect defects in the differentiation responses of the endometrium [68,69]. However, this approach is marred by high levels of inter- and intra-observer variation [70], poor inter-cycle association, and tissue fixation artefacts, which all limit the clinical usefulness of histological dating [71].

Electron microscopy allows assessment of endometrial ultrastructures, such as the epithelial cell membrane projections called 'pinopodes', which were considered to play a role in endometrial receptivity [72,73]. However, these enigmatic structures have now been found throughout the entire luteal phase and in early pregnancy [74] and their role remains unclear [75].

5.2. Molecular markers of endometrial receptivity

Several gene- and protein expression profiles of pre-receptive and receptive endometrium in natural [76–83] and ovarian stimulation cycles [84,82,85] have been performed in the last decade. Although all studies have identified numerous biomarkers, overlap in genes between studies is low because of different methods, techniques, patient characteristics and timing [86]. These studies suggest that endometrial receptivity is governed by expression of an evolutionarily conserved network of mediators. Thus far, it has been difficult to associate these factors with reproductive failure in women or to develop them as therapeutic targets. However within studies significant different expression profiles have been identified.

5.2.1. Gene expression

RIF patients show deregulated gene expression during the receptive phase compared to controls [16]. Pathways of cell cycle, Wnt signalling and cellular adhesion, are involved [16]. A recent study comparing implantation failures (IF), recurrent miscarriers (RM) and fertile controls (FC) showed different expression of 2126, 2477 and 2363 genes (IF vs. FC, RM vs. FC, IF vs RM resp.) [17]. Shared deregulated pathways involved DNA transcription and factors in the haematological system. IF showed high-deregulated gene expression in cell mediated immune response and nervous system development, while RM showed aberrant expression in humeral immune response and organ and muscle development [17]. Diaz-Gimeno et al. developed a predictor set of biomarkers for endometrial receptivity which was sensitive and specific for endometrial dating, but had a low specificity for detecting pathological classifications like RIF [83].

Studies focusing on p53 tumour suppressor gene, which regulates cell apoptosis, angiogenesis and is a potential mediator of pregnancy show significantly more homozygous genotypes in RIF patients [87–89].

MSX homeobox gene deletion in mice inhibits blastocyst implantation [90]. Micro-array analysis shows that these genes are down-regulated during the receptive window in humans [77,78,91], similar to what occurs in implantation in mice. These facts, together with failure to initiate implantation in uteri of MSX knock-out animals with a diapause (delayed implantation till favourable conditions are reached), suggest that the MSX genes play a role in the initiation of the window of implantation.

MicroRNA (miRNA) has a function in post-transcriptional regulation of gene expression by targeting mRNAs for degradation and/or translational repression [92,93], and thus have a role in the repression of protein expression. Recent findings suggest a role for miRNA in down regulating the expression of some cell cycle genes in secretory-phase endometrium [94]. In RIF patients 13 miRNAs were differently expressed, the genes involved play a role in Wnt signalling and cell cycle pathways and the formation of adhesion molecules [95].

5.2.2. Prostaglandins (PGs)

PGs are demonstrated to be crucial for successful embryo implantation [96,97]. Cyclooxygenases (COX-1 and COX-2) are the enzymes responsible for the synthesis of a variety of PGs, which are up-regulated by progesterone [98]. In a recent study, patients presenting with RIF expressed reduced levels of cPLA2 α and COX-2 compared with controls. In response to this deficiency, sPLA2-IIA was found to be overexpressed [99]. Prostaglandin synthesis therefore appears to be disrupted in patients with repeated IVF failure compared with fertile controls. Reduced levels of COX-2 and several prostaglandins were also detected in patients with unexplained recurrent miscarriage together with a lower level of VEGF [100]. The COX-derived signalling pathway possibly plays an important role in the successful embryo implantation.

5.2.3. Cell adhesion molecules (CAM)

The CAM family is composed of four members: integrins, selectins, cadherins, and immunoglobulins. These surface ligands, usually carbohydrate glycoproteins, mediate cell-to-cell adhesion. Expression of $\alpha V\beta 3$ integrin and its ligand osteopontin coincides with the opening of the WOI, was detected by immunohistochemistry on the endometrial luminal epithelial surface and is the first to interact with the trophoblast [101]. Aberrant $\alpha V\beta 3$ integrin expression pattern has been associated with unexplained infertility [102–104], and other gynaecological disorders like endometriosis [103] and polycystic ovarian syndrome [105]. Lower integrin mRNA level on day 21 is associated with a 50% lower implantation rate than normal levels [106,107].

Other luminal moieties include oligosaccharide ligands present on the luminal epithelial cells that attach to the embryonal L-selectin [108,109]. It appears that selectins contribute to the early events of embryo–maternal interactions [110]. E-cadherin is the most studied cadherin, with function of cell-to-cell adhesion. It is progesterone dependent via calcitonin expression and down-regulation probably plays a role in embryo invasion [110]. To date, the role of selectins or cadherin expression linked to (recurrent) implantation failure is poorly defined.

5.2.4. Mucins

In the endometrium, MUC-1-glycoprotein extends beyond the glycocalyx on the luminal epithelial endometrial layer and acts as a barrier for implantation. Human *in vitro* implantation models indicate that MUC-1 expression is increased during the receptive window [25] and lost at the site of embryo attachment [111]. Women with recurrent pregnancy loss (RPL) were shown to express reduced endometrial MUC-1, as compared with a normal group of patients [112–114]. MUC-1 is a highly polymorphic gene. An association between the allele size of MUC-1 and implantation failure was shown in 2001 [115]. Patients with implantation failure had significantly shorter extracellular chains that could make embryo apposition to the destined place in the endometrial lining difficult. In contrast, in 2004 a study compared the MUC-1 genotype of 10 fertile and 10 women with implantation failure and proposed that there was no association [116]. Also, in patients with a history of recurrent miscarriage no association was found [117].

5.2.5. Cytokines

Uterine Natural Killer cells (uNK) are the most abundant immune cells present in the endometrium. They secrete various cytokines important for adequate local immune regulation, angiogenesis, placental development, and establishment of pregnancy [33,118].

The autocrine and paracrine effects of the cytokine LIF, such as proliferation, differentiation and cell survival, made researchers investigate its role in implantation. In RIF patients a presumed role of LIF gene mutations has been investigated [119]. LIF secretion by human endometrial cells only weakly increased from the proliferative to secretory phase in patients with RIF and unexplained infertility [120]. However, a first randomized controlled clinical trial performed in 2009, in which recombinant LIF has been administered to patients with at least two failed ART cycles failed to demonstrate higher implantation rates in the intervention group [27].

Relevance of the IL-1 system in the implantation process was established by mouse experiments. Surprisingly, although IL-1 deficient mice were able to reach pregnancy, an intraperitoneal injection of IL-1ra at the appropriate time was enough to prevent blastocyst implantation. This was attributed to the down-regulation of critical integrins at the luminal epithelial surface [121]. Such a phenomenon appears to also occur in human. Indeed, supplementation of IL-1 in the culture media of endometrial epithelial cells (EECs) leads to the increase of integrin $\beta 3$ expression and thereby to enhanced blastocyst implantation [122].

Expression of IL-15 and IL-18 has been shown to be different in patients with failed implantation after IVF/ICSI compared with fertile controls and both correlate with local uNK recruitment and angiogenesis [18].

IL-6 deficient mice showed reduced implantation sites and reduced fertility. Abnormal expression of IL-6 in late secretory phase was reported in patients with RM [123].

Studies on IL-11 and IL-11 α revealed that expression of these markers were lower in the endometrial biopsies of RM patients compared to fertile controls [124], suggesting a role in endometrial decidualization.

5.2.6. Others

Recent analysis of mid-secretory endometrial samples revealed that increased serum- and glucocorticoid- inducible kinase SGK1, a kinase involved in epithelial ion transport and cell survival, interferes with embryo implantation in endometrial surface epithelium, leading to infertility [125,126]. However, implantation was not impaired in Sgk1-deficient mice, although there was evidence of bleeding and inflammation at the fetomaternal interface in early pregnancy and subsequent foetal demise [126]. Another recent study showed endometrial placental growth factor (PLGF) expression corresponded to the hysteroscopic appearance of the endometrium, and showed a lower expression in patients with implantation failure [127].

6. Clinical challenges

While there has been progress in our knowledge of the aetiologies of implantation failure, a full understanding of *in-vivo* determinants of human implantation has been hampered by a lack of appropriate models. However, recent developments in diagnostic techniques and molecular tools are now opening the ‘black box’ of implantation failure. A key step has been the development of less invasive means of assessing of endometrial receptivity. Non-disruptive analysis of the endometrial environment at the moment of embryo replacement can be performed by analysis of endometrial secretions [10]. Endometrial secretion aspiration can be carried out immediately prior to embryo transfer without affecting implantation rates [128]. Proteomic analysis by mass spectrometry showed different protein expression patterns during the prereceptive and receptive phases [129]. A multiplex immunoassay for 17 regulators of implantation showed a cytokine profile conducive to clinical pregnancy [10]. While 2D-DiGE analysis of the human endometrial secretome revealed differences between receptive states in fertile and infertile women [130].

In favour of endometrial biopsy a number of studies showed that endometrial local injury in a cycle prior to IVF/ICSI treatment resulted in increased implantation and pregnancy rates [131–133]. Possible explanation could be induced decidualisation and/or the wound healing effect, accompanied with influx of immune cells, secretion of inflammatory mediators and growth factors, including LIF, IL-11 and HB-EGF [134].

7. Mother and embryo

The technical and ethical obstacles to study human implantation *in vivo* have necessitated the development of *in vitro* models to examine embryo–endometrial interactions [30,28,29]. These models can provide a key role in elucidating role of the embryo or the endometrium in initiating or indeed terminating the preimplantation dialogue. Using a human co-culture model, soluble implantation factors were determined in case of developing and arrested embryos by multiplex immunoassay [135]. The model consisted of decidualizing endometrial stromal cells (ESCs) and single hatched blastocysts. Over a 3-day co-culture period, 25% of embryos showed development and the remainder arrested. Surprisingly, the presence of a developing embryo had no significant effect on decidual secretions while an

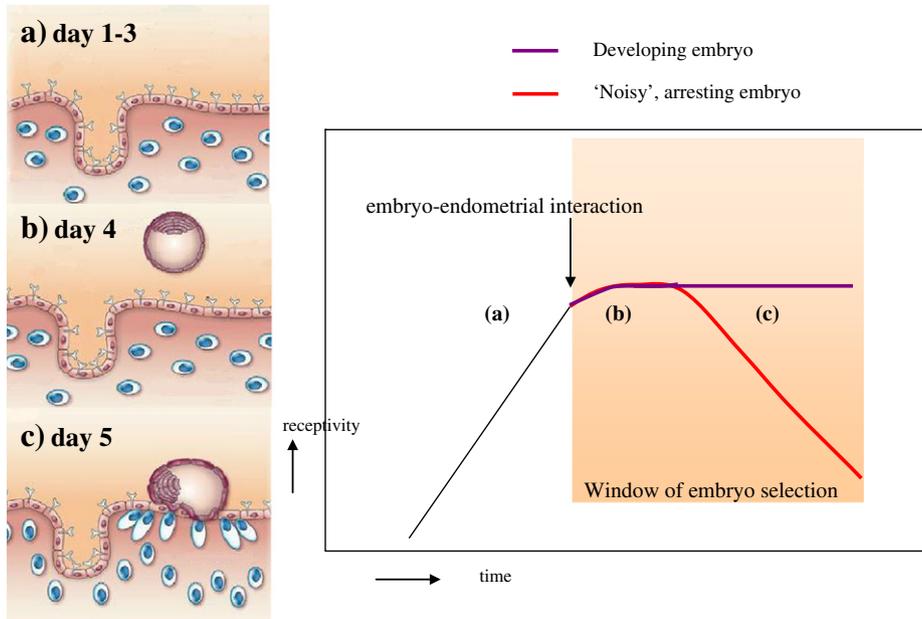


Fig. 2. Mechanisms of embryo selection derived from in vitro models of embryo–endometrial interaction.

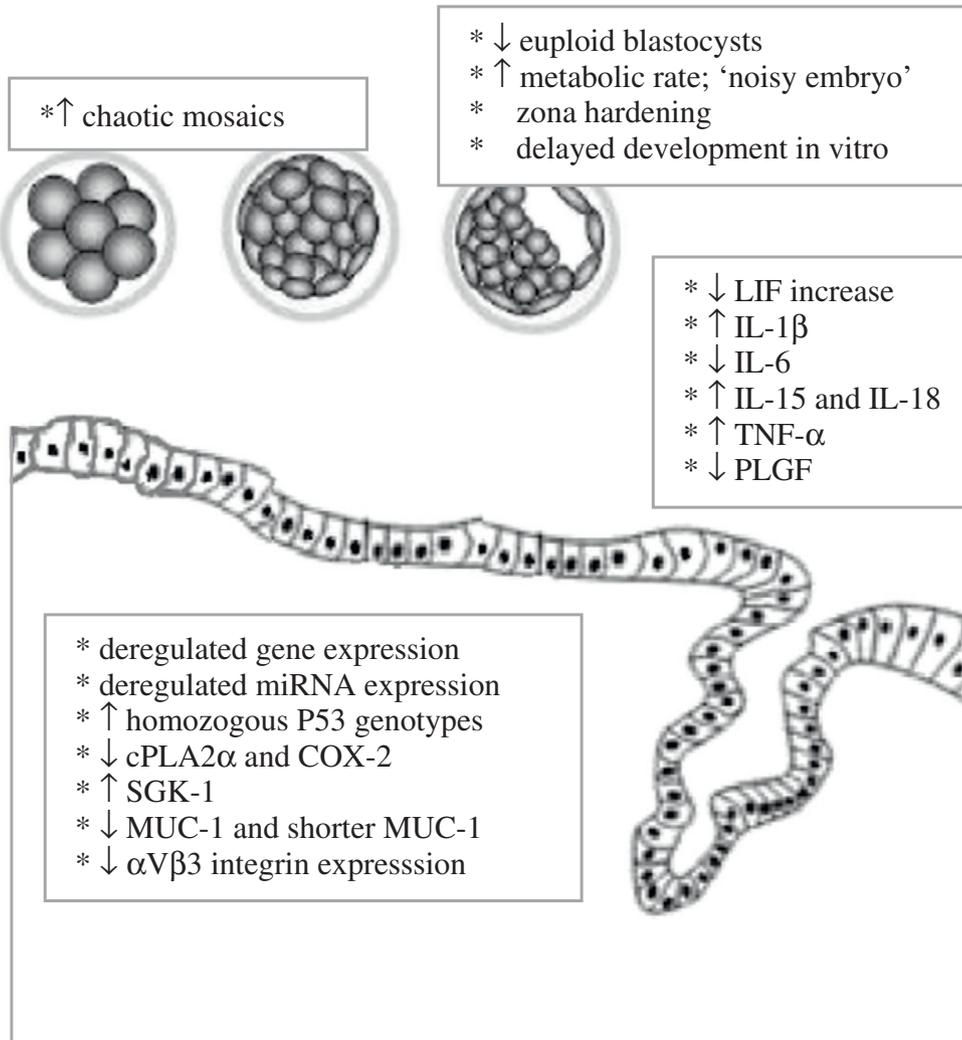


Fig. 3. Embryonal and endometrial factors of human implantation associated with (recurrent) implantation failure.

arresting embryo triggered a strong response (selective inhibition of IL-1b, -6, -10, -17, -18, eotaxin, and HB-EGF secretion). Co-cultures with undifferentiated ESCs showed no effect by the presence of a developing or arresting embryo [135]. These results suggest that the differentiated endometrium can differentiate between good and bad quality embryos and reject the latter; a phenomenon which has been termed natural embryo selection (Fig. 2) [32].

It has been proposed that RPL could be caused by failure of natural embryo quality control [136] whereas RIF patients have a selection mechanism that inappropriately rejects good quality embryos. These mechanisms need further attention in new developed models.

8. Conclusion

Successful implantation of a good quality human embryo in a receptive endometrium requires a remarkable and complex collaboration of factors.

Studies on gene- and protein expression profiles using mRNA micro arrays have led to the identification of numerous putative endometrial biomarkers of both successful and unsuccessful implantations (Fig. 3; factors associated with RIF). With the development of bioinformatics technologies and emerging databases it should become possible to identify genes and proteins that are predictors of endometrial receptivity and pregnancy outcome of clinical value.

Studies using in vitro models suggest that the decidualized endometrium is capable of selecting good quality embryos and, more importantly, reject the incompetent embryos. It has been proposed that this process could be impaired in patients with RPL and RIF.

To conclude future research should focus on integrating data from gene and protein expression studies in endometrial biopsies and secretions to determine markers with clinical significance. Next to this, in vitro models appear a promising mode of interrogating embryo-endometrial interactions to investigate possible interventions designed to reduce RIF and pregnancy failure.

References

- J.R. Marshall, C.B. Hammond, G.T. Ross, A. Jacobson, P. Rayford, W.D. Odell, Plasma and urinary chorionic gonadotropin during early human pregnancy, *Obstet. Gynecol.* 32 (1968) 760–764.
- E.G. Armstrong, P.H. Ehrlich, S. Birken, J.P. Schlatterer, E. Siris, W.C. Hembree, R.E. Canfield, Use of a highly sensitive and specific immunoradiometric assay for detection of human chorionic gonadotropin in urine of normal, nonpregnant, and pregnant individuals, *J. Clin. Endocrinol. Metab.* 59 (1984) 867–874.
- R.E. Canfield, J.F. O'Connor, S. Birken, A. Krichevsky, A.J. Wilcox, Development of an assay for a biomarker of pregnancy and early fetal loss, *Environ. Health Perspect.* 74 (1987) 57–66.
- J. Rinehart, Recurrent implantation failure: definition, *J. Assist. Reprod. Genet.* 24 (2007) 284–287.
- A.J. Wilcox, C.R. Weinberg, J.F. O'Connor, D.D. Baird, J.P. Schlatterer, R.E. Canfield, E.G. Armstrong, B.C. Nisula, Incidence of early loss of pregnancy, *N. Engl. J. Med.* 319 (1988) 189–194.
- X. Wang, C. Chen, L. Wang, D. Chen, W. Guang, J. French, Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study, *Fertil. Steril.* 79 (2003) 577–584.
- Y.E. Koot, C.M. Boomsma, M.J. Eijkemans, E.G. Lentjes, N.S. Macklon, Recurrent pre-clinical pregnancy loss is unlikely to be a 'cause' of unexplained infertility, *Hum. Reprod.* 26 (2011) 2636–2641.
- A.J. Wilcox, D.D. Baird, C.R. Weinberg, E.G. Armstrong, P.I. Musey, R.E. Wehmann, R.E. Canfield, The use of biochemical assays in epidemiologic studies of reproduction, *Environ. Health Perspect.* 75 (1987) 29–35.
- R.J. Norman, M. Menabawey, C. Lowings, R.H. Buck, T. Chard, Relationship between blood and urine concentrations of intact human chorionic gonadotropin and its free subunits in early pregnancy, *Obstet. Gynecol.* 69 (1987) 590–593.
- C.M. Boomsma, A. Kavelaars, M.J. Eijkemans, E.G. Lentjes, B.C. Fauser, C.J. Heijnen, N.S. Macklon, Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF, *Hum. Reprod.* 24 (2009) 1427–1435.
- A.R. Thornhill, C.E. Die-Smulders, J.P. Geraedts, J.C. Harper, G.L. Harton, S.A. Lavery, C. Moutou, M.D. Robinson, A.G. Schmutzler, P.N. Scriven, K.D. Sermon, L. Wilton, ESHRE PGD Consortium 'Best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)', *Hum. Reprod.* 20 (2005) 35–48.
- R.J. Paulson, I.E. Hatch, R.A. Lobo, M.V. Sauer, Cumulative conception and live birth rates after oocyte donation: implications regarding endometrial receptivity, *Hum. Reprod.* 12 (1997) 835–839.
- A.K. Schroder, A. Katalinic, K. Diedrich, M. Ludwig, Cumulative pregnancy rates and drop-out rates in a German IVF programme: 4102 cycles in 2130 patients, *Reprod. Biomed. Online* 8 (2004) 600–606.
- B.S. Shapiro, K.S. Richter, D.C. Harris, S.T. Daneshmand, Dramatic declines in implantation and pregnancy rates in patients who undergo repeated cycles of in vitro fertilization with blastocyst transfer after one or more failed attempts, *Fertil. Steril.* 76 (2001) 538–542.
- C. Blockeel, V. Schutyser, V.A. De, W. Verpoest, V.M. De, C. Staessen, P. Haentjens, E.J. Van Der, P. Devroey, Prospectively randomized controlled trial of PGS in IVF/ICSI patients with poor implantation, *Reprod. Biomed. Online* 17 (2008) 848–854.
- M. Koler, H. Achache, A. Tsafirir, Y. Smith, A. Revel, R. Reich, Disrupted gene pattern in patients with repeated in vitro fertilization (IVF) failure, *Hum. Reprod.* 24 (2009) 2541–2548.
- N. Ledee, C. Munaut, J. Aubert, V. Serazin, M. Rahmati, G. Chaouat, O. Sandra, J.M. Foidart, Specific and extensive endometrial deregulation is present before conception in IVF/ICSI repeated implantation failures (IF) or recurrent miscarriages, *J. Pathol.* 225 (2011) 554–564.
- N. Lédée, M. Petitbarat, M. Rahmati, S. Dubanchet, G. Chaouat, O. Sandra, S. Perrier-d'Hauterive, C. Munaut, J.M. Foidart, New pre-conception immune biomarkers for clinical practice: interleukin-18, interleukin-15 and TWEAK on the endometrial side, G-CSF on the follicular side, *J. Reprod. Immunol.* 88 (2011) 118–123.
- C.F.D.C.a.P. CDC, Assisted reproductive technologies success rate, CDC. (2008 B.C.).
- J.D.T.B.-P.E.A.S. Yueping Alex Wang, Assisted reproduction technology in Australia and New Zealand 2006, AIHW. number 12 (08).
- M.J. de, V. Goossens, S. Bhattacharya, J.A. Castilla, A.P. Ferraretti, V. Korsk, M. Kupka, K.G. Nygren, A.A. Nyboe, Assisted reproductive technology in Europe, 2006: results generated from European registers by ESHRE, *Hum. Reprod.* 25 (2010) (2006) 1851–1862.
- H. Wang, S.K. Dey, Roadmap to embryo implantation: clues from mouse models, *Nat. Rev. Genet.* 7 (2006) 185–199.
- C.L. Stewart, P. Kaspar, L.J. Brunet, H. Bhatt, I. Gadi, F. Kontgen, S.J. Abbondanzo, Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor, *Nature* 359 (1992) 76–79.
- G.A. Surveyor, S.J. Gendler, L. Pemberton, S.K. Das, I. Chakraborty, J. Julian, R.A. Pimental, C.C. Wegner, S.K. Dey, D.D. Carson, Expression and steroid hormonal control of Muc-1 in the mouse uterus, *Endocrinology* 136 (1995) 3639–3647.
- N.A. Hey, R.A. Graham, M.W. Seif, J.D. Aplin, The polymorphic epithelial mucin MUC1 in human endometrium is regulated with maximal expression in the implantation phase, *J. Clin. Endocrinol. Metab.* 78 (1994) 337–342.
- E.B. Cullinan, S.J. Abbondanzo, P.S. Anderson, J.W. Pollard, B.A. Lessey, C.L. Stewart, Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation, *Proc. Natl. Acad. Sci. U. S. A.* 93 (1996) 3115–3120.
- P.R. Brinsden, V. Alam, M.B. de, P. Engrand, Recombinant human leukemia inhibitory factor does not improve implantation and pregnancy outcomes after assisted reproductive techniques in women with recurrent unexplained implantation failure, *Fertil. Steril.* 91 (2009) 1445–1447.
- J. Carver, K. Martin, I. Spyropoulou, D. Barlow, I. Sargent, H. Mardon, An in-vitro model for stromal invasion during implantation of the human blastocyst, *Hum. Reprod.* 18 (2003) 283–290.
- S. Hombach-Klonisch, A. Kehlen, P.A. Fowler, B. Huppertz, J.F. Jugert, G. Bischoff, E. Schluter, J. Buchmann, T. Klonisch, Regulation of functional steroid receptors and ligand-induced responses in telomerase-immortalized human endometrial epithelial cells, *J. Mol. Endocrinol.* 34 (2005) 517–534.
- G. Teklenburg, N.S. Macklon, Review: in vitro models for the study of early human embryo-endometrium interactions, *Reprod. Sci.* 16 (2009) 811–818.
- M. Salker, G. Teklenburg, M. Molokhia, S. Lavery, G. Trew, T. Aojanepong, H.J. Mardon, A.U. Lokugamage, R. Rai, C. Landles, B.A. Roelen, S. Quenby, E.W. Kuijk, A. Kavelaars, C.J. Heijnen, L. Regan, N.S. Macklon, J.J. Brosens, Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss, *PLoS One* 5 (2010) e10287-.
- G. Teklenburg, M. Salker, C. Heijnen, N.S. Macklon, J.J. Brosens, The molecular basis of recurrent pregnancy loss: impaired natural embryo selection, *Mol. Hum. Reprod.* 16 (2010) 886–895.
- G. Chaouat, S. Dubanchet, N. Ledee, Cytokines: Important for implantation? *J. Assist. Reprod. Genet.* 24 (2007) 491–505.
- B. Urman, K. Yakin, B. Balaban, Recurrent implantation failure in assisted reproduction: how to counsel and manage. A General considerations and treatment options that may benefit the couple, *Reprod. Biomed. Online* 11 (2005) 371–381.
- S. Munne, Preimplantation genetic diagnosis of structural abnormalities, *Mol. Cell. Endocrinol.* 183 (Suppl. 1) (2001) S55–S58.
- J.D. Delhanty, Mechanisms of aneuploidy induction in human oogenesis and early embryogenesis, *Cytogenet. Genome Res.* 111 (2005) 237–244.
- E. Vanneste, T. Voet, C.C. Le, M. Ampe, P. Konings, C. Melotte, S. Debrock, M. Amyere, N. Vikkula, F. Schuit, J.P. Fryns, G. Verbeke, T. D'Hooghe, Y. Moreau, J.R. Vermeesch, Chromosome instability is common in human cleavage-stage embryos, *Nat. Med.* 15 (2009) 577–583.
- L. Wilton, Preimplantation genetic diagnosis for aneuploidy screening in early human embryos: a review, *Prenat. Diagn.* 22 (2002) 512–518.

- [39] C. Gutierrez-Mateo, P. Colls, J. Sanchez-Garcia, T. Escudero, R. Prates, K. Ketterson, D. Wells, S. Munne, Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos, *Fertil. Steril.* 95 (2011) 953–958.
- [40] E.B. Baart, E. Martini, I. van d.B., N.S. Macklon, R.J. Galjaard, B.C. Fauser, O.D. Van, Preimplantation genetic screening reveals a high incidence of aneuploidy and mosaicism in embryos from young women undergoing IVF, *Hum. Reprod.* 21 (2006) 223–233.
- [41] A. Mantzouratou, A. Mania, E. Fragouli, L. Xanthopoulou, S. Tashkandi, K. Fordham, D.M. Ranieri, A. Doshi, S. Nuttall, J.C. Harper, P. Serhal, J.D. Delhanty, Variable aneuploidy mechanisms in embryos from couples with poor reproductive histories undergoing preimplantation genetic screening, *Hum. Reprod.* 22 (2007) 1844–1853.
- [42] M.A. Santos, G. Teklenburg, N.S. Macklon, O.D. Van, G.H. Schuring-Blom, P.J. Krijtenburg, J. de Vreeden-Elbertse, B.C. Fauser, E.B. Baart, The fate of the mosaic embryo: chromosomal constitution and development of Day 4, 5 and 8 human embryos, *Hum. Reprod.* 25 (2010) 1916–1926.
- [43] L.E. Northrop, N.R. Treff, B. Levy, R.T. Scott Jr., SNP microarray-based 24 chromosome aneuploidy screening demonstrates that cleavage-stage FISH poorly predicts aneuploidy in embryos that develop to morphologically normal blastocysts, *Mol. Hum. Reprod.* 16 (2010) 590–600.
- [44] L. Voullaire, V. Collins, T. Callaghan, J. McBain, R. Williamson, L. Wilton, High incidence of complex chromosome abnormality in cleavage embryos from patients with repeated implantation failure, *Fertil. Steril.* 87 (2007) 1053–1058.
- [45] J.C. Harper, S.B. Sengupta, Preimplantation genetic diagnosis: State of the ART 2011, *Hum. Genet.* 131 (2012) 175–186.
- [46] F.D. Houghton, J.A. Hawkhead, P.G. Humpherson, J.E. Hogg, A.H. Balen, A.J. Rutherford, H.J. Leese, Non-invasive amino acid turnover predicts human embryo developmental capacity, *Hum. Reprod.* 17 (2002) 999–1005.
- [47] D.R. Brison, F.D. Houghton, D. Falconer, S.A. Roberts, J. Hawkhead, P.G. Humpherson, B.A. Lieberman, H.J. Leese, Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover, *Hum. Reprod.* 19 (2004) 2319–2324.
- [48] H.J. Leese, C.G. Baumann, D.R. Brison, T.G. McEvoy, R.G. Sturmey, Metabolism of the viable mammalian embryo: quietness revisited, *Mol. Hum. Reprod.* 14 (2008) 667–672.
- [49] H.M. Picton, K. Elder, F.D. Houghton, J.A. Hawkhead, A.J. Rutherford, J.E. Hogg, H.J. Leese, S.E. Harris, Association between amino acid turnover and chromosome aneuploidy during human preimplantation embryo development in vitro, *Mol. Hum. Reprod.* 16 (2010) 557–569.
- [50] F. Dominguez, B. Gadea, F.J. Esteban, J.A. Horcajadas, A. Pellicer, C. Simon, Comparative protein-profile analysis of implanted versus non-implanted human blastocysts, *Hum. Reprod.* 23 (2008) 1993–2000.
- [51] S.S. Cortezzi, J.S. Garcia, C.R. Ferreira, D.P. Braga, R.C. Figueira, A. Iaconelli Jr., G.H. Souza, E. Borges Jr., M.N. Eberlin, Secretome of the preimplantation human embryo by bottom-up label-free proteomics, *Anal. Bioanal. Chem.* 401 (2011) 1331–1339.
- [52] J. Cohen, C. Elsner, H. Kort, H. Malter, J. Massey, M.P. Mayer, K. Wiemer, Impairment of the hatching process following IVF in the human and improvement of implantation by assisting hatching using micromanipulation, *Hum. Reprod.* 5 (1990) 7–13.
- [53] M. Zhao, J. Dean, The zona pellucida in folliculogenesis, fertilization and early development, *Rev. Endocr. Metab. Disord.* 3 (2002) 19–26.
- [54] R.J. Cole, Cinemicrographic observations on the trophoblast and zona pellucida of the mouse blastocyst, *J. Embryol. Exp. Morphol.* 17 (1967) 481–490.
- [55] I. Demeestere, P. Barlow, F. Leroy, Hardening of zona pellucida of mouse oocytes and embryos in vivo and in vitro, *Int. J. Fertil. Womens Med.* 42 (1997) 219–222.
- [56] J. Carroll, H. Depypere, C.D. Matthews, Freeze-thaw-induced changes of the zona pellucida explains decreased rates of fertilization in frozen-thawed mouse oocytes, *J. Reprod. Fertil.* 90 (1990) 547–553.
- [57] J.A. Horcajadas, P. Minguez, J. Dopazo, F.J. Esteban, F. Dominguez, L.C. Giudice, A. Pellicer, C. Simon, Controlled ovarian stimulation induces a functional genomic delay of the endometrium with potential clinical implications, *J. Clin. Endocrinol. Metab.* 93 (2008) 4500–4510.
- [58] M.I. Hsu, J. Mayer, M. Aronshon, S. Lanzendorf, S. Muasher, P. Kolm, S. Oehninger, Embryo implantation in vitro fertilization and intracytoplasmic sperm injection: impact of cleavage status, morphology grade, and number of embryos transferred, *Fertil. Steril.* 72 (1999) 679–685.
- [59] W.P. Martins, I.A. Rocha, R.A. Ferriani, C.O. Nastri, Assisted hatching of human embryos: a systematic review and meta-analysis of randomized controlled trials, *Hum. Reprod. Update* 17 (2011) 438–453.
- [60] S. Das, D. Blake, C. Farquhar, M.M. Seif, Assisted hatching on assisted conception (IVF and ICSI), *Cochrane Database Syst. Rev.* (2009) CD001894.
- [61] B. Gellersen, I.A. Brosens, J.J. Brosens, Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives, *Semin. Reprod. Med.* 25 (2007) 445–453.
- [62] M. Takano, Z. Lu, T. Goto, L. Fusi, J. Higham, J. Francis, A. Withey, J. Hardt, B. Cloke, A.V. Stavropoulou, O. Ishihara, E.W. Lam, T.G. Unterman, J.J. Brosens, J.J. Kim, Transcriptional cross talk between the forkhead transcription factor forkhead box O1A and the progesterone receptor coordinates cell cycle regulation and differentiation in human endometrial stromal cells, *Mol. Endocrinol.* 21 (2007) 2334–2349.
- [63] S.K. Dey, H. Lim, S.K. Das, J. Reese, B.C. Paria, T. Daikoku, H. Wang, Molecular cues to implantation, *Endocr. Rev.* 25 (2004) 341–373.
- [64] P.A. Bergh, D. Navot, The impact of embryonic development and endometrial maturity on the timing of implantation, *Fertil. Steril.* 58 (1992) 537–542.
- [65] J.J. Brosens, B. Gellersen, Death or survival—progesterone-dependent cell fate decisions in the human endometrial stroma, *J. Mol. Endocrinol.* 36 (2006) 389–398.
- [66] E.M. Ramsey, M.L. Houston, J.W. Harris, Interactions of the trophoblast and maternal tissues in three closely related primate species, *Am. J. Obstet. Gynecol.* 124 (1976) 647–652.
- [67] J.J. Brosens, R. Pijnenborg, I.A. Brosens, The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature, *Am. J. Obstet. Gynecol.* 187 (2002) 1416–1423.
- [68] R.W. Noyes, A.T. Hertig, J. Rock, Dating the endometrial biopsy, *Fertil. Steril.* 1 (1950) 3–25.
- [69] R.W. Noyes, J.O. Haman, Accuracy of endometrial dating; correlation of endometrial dating with basal body temperature and menses, *Fertil. Steril.* 4 (1953) 504–517.
- [70] M.J. Murray, W.R. Meyer, R.J. Zaino, B.A. Lessey, D.B. Novotny, K. Ireland, D. Zeng, M.A. Fritz, A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women, *Fertil. Steril.* 81 (2004) 1333–1343.
- [71] K. Diedrich, B.C. Fauser, P. Devroey, G. Griesinger, The role of the endometrium and embryo in human implantation, *Hum. Reprod. Update* 13 (2007) 365–377.
- [72] U. Tin-Ley, A. Sjogren, L. Nilsson, L. Hamberger, J.F. Larsen, T. Horn, Presence of uterine pinopodes at the embryo-endometrial interface during human implantation in vitro, *Hum. Reprod.* 14 (1999) 515–520.
- [73] C. Isaac, J.W. Pollard, U.T. Meier, Intranuclear endoplasmic reticulum induced by Nopp 140 mimics the nuclear channel system of human endometrium, *J. Cell Sci.* 114 (2001) 4253–4264.
- [74] C. Quinn, E. Ryan, E.A. Claessens, E. Greenblatt, P. Hawrylyshyn, B. Cruickshank, T. Hannam, C. Dunk, R.F. Casper, The presence of pinopodes in the human endometrium does not delineate the implantation window, *Fertil. Steril.* 87 (2007) 1015–1021.
- [75] C.E. Quinn, R.F. Casper, Pinopodes: a questionable role in endometrial receptivity, *Hum. Reprod. Update* 15 (2009) 229–236.
- [76] D.D. Carson, E. Lagow, A. Thathiah, R. Al-Shami, M.C. Farach-Carson, M. Vernon, L. Yuan, M.A. Fritz, B. Lessey, Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening, *Mol. Hum. Reprod.* 8 (2002) 871–879.
- [77] A. Riesewijk, J. Martin, O.R. van, J.A. Horcajadas, J. Polman, A. Pellicer, S. Mosselman, C. Simon, Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology, *Mol. Hum. Reprod.* 9 (2003) 253–264.
- [78] S. Mirkin, M. Arslan, D. Churikov, A. Corica, J.I. Diaz, S. Williams, S. Bocca, S. Oehninger, In search of candidate genes critically expressed in the human endometrium during the window of implantation, *Hum. Reprod.* 20 (2005) 2104–2117.
- [79] J. Li, Z. Tan, M.T. Li, Y.L. Liu, Q. Liu, X.F. Gu, J.Z. Zhou, G.L. Zhuang, Study of altered expression of annexin IV and human endometrial receptivity, *Zhonghua Fu Chan Ke Za Zhi* 41 (2006) 803–805.
- [80] S. Talbi, A.E. Hamilton, K.C. Vo, S. Tulac, M.T. Overgaard, C. Dosiou, S.N. Le, C.N. Nezhat, R. Kempson, B.A. Lessey, N.R. Nayak, L.C. Giudice, Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women, *Endocrinology* 147 (2006) 1097–1121.
- [81] F. Dominguez, T. Garrido-Gomez, J.A. Lopez, E. Camafeita, A. Quinonero, A. Pellicer, C. Simon, Proteomic analysis of the human receptive versus non-receptive endometrium using differential in-gel electrophoresis and MALDI-MS unveils stathmin 1 and annexin A2 as differentially regulated, *Hum. Reprod.* 24 (2009) 2607–2617.
- [82] D. Haouzi, S. Assou, K. Mahmoud, S. Tondeur, T. Reme, B. Hedon, V.J. De, S. Hamamah, Gene expression profile of human endometrial receptivity: comparison between natural and stimulated cycles for the same patients, *Hum. Reprod.* 24 (2009) 1436–1445.
- [83] P. Az-Gimeno, J.A. Horcajadas, J.A. Martinez-Conejero, F.J. Esteban, P. Alama, A. Pellicer, C. Simon, A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature, *Fertil. Steril.* 95 (2011) 50–60 (60).
- [84] N.S. Macklon, M.H. van der Gaast, A. Hamilton, B.C. Fauser, L.C. Giudice, The impact of ovarian stimulation with recombinant FSH in combination with GnRH antagonist on the endometrial transcriptome in the window of implantation, *Reprod. Sci.* 15 (2008) 357–365.
- [85] D. Haouzi, S. Assou, C. Dechanet, T. Anahory, H. Dechaud, V.J. De, S. Hamamah, Controlled ovarian hyperstimulation for in vitro fertilization alters endometrial receptivity in humans: protocol effects, *Biol. Reprod.* 82 (2010) 679–686.
- [86] D. Haouzi, H. Dechaud, S. Assou, V.J. De, S. Hamamah, Insights into human endometrial receptivity from transcriptomic and proteomic data, *Reprod. Biomed. Online* 24 (2012) 23–34.
- [87] C. Goodman, R.S. Jeyendran, C.B. Coulam, P53 tumor suppressor factor, plasminogen activator inhibitor, and vascular endothelial growth factor gene polymorphisms and recurrent implantation failure, *Fertil. Steril.* 92 (2009) 494–498.
- [88] H.J. Kang, Z. Feng, Y. Sun, G. Atwal, M.E. Murphy, T.R. Rebbeck, Z. Rosenwaks, A.J. Levine, W. Hu, Single-nucleotide polymorphisms in the p53 pathway regulate fertility in humans, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 9761–9766.
- [89] R.D. Firoozabadi, N. Ghasemi, M.A. Rozbahani, N. Tabibnejad, Association of p53 polymorphism with ICSI/IVF failure and recurrent pregnancy loss, *Aust. N. Z. J. Obstet. Gynaecol.* 49 (2009) 216–219.
- [90] T. Daikoku, J. Cha, X. Sun, S. Tranguch, H. Xie, T. Fujita, Y. Hirota, J. Lydon, F. Demayo, R. Maxson, S.K. Dey, Conditional Deletion of MSX Homeobox Genes in the Uterus Inhibits Blastocyst Implantation by Altering Uterine Receptivity, *Dev. Cell* 21 (2011) 1014–1025.

- [91] L.C. Kao, S. Tulac, S. Lobo, B. Imani, J.P. Yang, A. Germeyer, K. Osteen, R.N. Taylor, B.A. Lessey, L.C. Giudice, Global gene profiling in human endometrium during the window of implantation, *Endocrinology* 143 (2002) 2119–2138.
- [92] D.P. Bartel, C.Z. Chen, Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs, *Nat. Rev. Genet.* 5 (2004) 396–400.
- [93] L.P. Lim, N.C. Lau, P. Garrett-Engle, A. Grimson, J.M. Schelter, J. Castle, D.P. Bartel, P.S. Linsley, J.M. Johnson, Microarray analysis shows that some microRNAs down-regulate large numbers of target mRNAs, *Nature* 433 (2005) 769–773.
- [94] S. Kuokkanen, B. Chen, L. Ojalvo, L. Benard, N. Santoro, J.W. Pollard, Genomic profiling of microRNAs and messenger RNAs reveals hormonal regulation in microRNA expression in human endometrium, *Biol. Reprod.* 82 (2010) 791–801.
- [95] A. Revel, H. Achache, J. Stevens, Y. Smith, R. Reich, MicroRNAs are associated with human embryo implantation defects, *Hum. Reprod.* 26 (2011) 2830–2840.
- [96] H. Song, H. Lim, B.C. Paria, H. Matsumoto, L.L. Swift, J. Morrow, J.V. Bonventre, S.K. Dey, Cytosolic phospholipase A2alpha is crucial [correction of A2alpha deficiency is crucial] for 'on-time' embryo implantation that directs subsequent development, *Development* 129 (2002) 2879–2889.
- [97] X. Ye, K. Hama, J.J. Contos, B. Anliker, A. Inoue, M.K. Skinner, H. Suzuki, T. Amano, G. Kennedy, H. Arai, J. Aoki, J. Chun, LPA3-mediated lysophosphatidic acid signalling in embryo implantation and spacing, *Nature* 435 (2005) 104–108.
- [98] Y. Kotani, A. Iwase, H. Ando, S. Mizutani, Oxytocin-induced prostaglandin E2 (PGE2) synthesis is regulated by progesterone via oxytocinase in Ishikawa cells, *Horm. Metab. Res.* 37 (2005) 4–9.
- [99] H. Achache, A. Tsafir, D. Prus, R. Reich, A. Revel, Defective endometrial prostaglandin synthesis identified in patients with repeated implantation failure undergoing in vitro fertilization, *Fertil. Steril.* 94 (2010) 1271–1278.
- [100] Y. Wang, A.M. Zhao, Q.D. Lin, Role of cyclooxygenase-2 signaling pathway dysfunction in unexplained recurrent spontaneous abortion, *Chin. Med. J. (Engl.)* 123 (2010) 1543–1547.
- [101] K.B. Apparao, M.J. Murray, M.A. Fritz, W.R. Meyer, A.F. Chambers, P.R. Truong, B.A. Lessey, Osteopontin and its receptor alphavbeta(3) integrin are coexpressed in the human endometrium during the menstrual cycle but regulated differentially, *J. Clin. Endocrinol. Metab.* 86 (2001) 4991–5000.
- [102] L.D. Klentzeris, J.N. Bulmer, L.K. Trejdosiewicz, L. Morrison, I.D. Cooke, Beta-1 integrin cell adhesion molecules in the endometrium of fertile and infertile women, *Hum. Reprod.* 8 (1993) 1223–1230.
- [103] B.A. Lessey, A.J. Castelbaum, S.W. Sawin, J. Sun, Integrins as markers of uterine receptivity in women with primary unexplained infertility, *Fertil. Steril.* 63 (1995) 535–542.
- [104] C. Tei, T. Maruyama, N. Kuji, T. Miyazaki, M. Mikami, Y. Yoshimura, Reduced expression of alphavbeta3 integrin in the endometrium of unexplained infertility patients with recurrent IVF-ET failures: improvement by danazol treatment, *J. Assist. Reprod. Genet.* 20 (2003) 13–20.
- [105] K.B. Apparao, L.P. Lovely, Y. Gui, R.A. Lininger, B.A. Lessey, Elevated endometrial androgen receptor expression in women with polycystic ovarian syndrome, *Biol. Reprod.* 66 (2002) 297–304.
- [106] K. Thomas, A. Thomson, S. Wood, C. Kingsland, G. Vince, I. Lewis-Jones, Endometrial integrin expression in women undergoing in vitro fertilization and the association with subsequent treatment outcome, *Fertil. Steril.* 80 (2003) 502–507.
- [107] A. Revel, A. Helman, M. Koler, A. Shushan, O. Goldshmidt, E. Zcharia, H. Aingorn, I. Vlodayk, Heparanase improves mouse embryo implantation, *Fertil. Steril.* 83 (2005) 580–586.
- [108] O.D. Genbacev, A. Prakobphol, R.A. Foulk, A.R. Krtolica, D. Ilic, M.S. Singer, Z.Q. Yang, L.L. Kiessling, S.D. Rosen, S.J. Fisher, Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface, *Science* 299 (2003) 405–408.
- [109] A.T. Fazleabas, J.J. Kim, Development. What makes an embryo stick? *Science* 299 (2003) 355–356.
- [110] H. Achache, A. Revel, Endometrial receptivity markers, the journey to successful embryo implantation, *Hum. Reprod. Update* 12 (2006) 731–746.
- [111] M. Meseguer, J.D. Aplin, P. Caballero-Campo, J.E. O'Connor, J.C. Martin, J. Remohi, A. Pellicer, C. Simon, Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst, *Biol. Reprod.* 64 (2001) 590–601.
- [112] E. Serle, J.D. Aplin, T.C. Li, M.A. Warren, R.A. Graham, M.W. Seif, I.D. Cooke, Endometrial differentiation in the peri-implantation phase of women with recurrent miscarriage: a morphological and immunohistochemical study, *Fertil. Steril.* 62 (1994) 989–996.
- [113] J.D. Aplin, N.A. Hey, T.C. Li, MUC1 as a cell surface and secretory component of endometrial epithelium: reduced levels in recurrent miscarriage, *Am. J. Reprod. Immunol.* 35 (1996) 261–266.
- [114] N.A. Hey, T.C. Li, P.L. Devine, R.A. Graham, H. Saravelos, J.D. Aplin, MUC1 in secretory phase endometrium: expression in precisely dated biopsies and flushings from normal and recurrent miscarriage patients, *Hum. Reprod.* 10 (1995) 2655–2662.
- [115] A.W. Horne, J.O. White, R.A. Margara, R. Williams, R.M. Winston, E. Lalani, MUC 1: a genetic susceptibility to infertility? *Lancet* 357 (2001) 1336–1337.
- [116] L.R. Goulart, G.S. Vieira, L. Martelli, J. Inacio, I.M. Goulart, J.G. Franco Jr., Is MUC1 polymorphism associated with female infertility? *Reprod. Biomed. Online* 8 (2004) 477–482.
- [117] D.B. Dentillo, F.R. Souza, J. Meola, G.S. Vieira, M.E. Yazlle, L.R. Goulart, L. Martelli, No evidence of association of MUC-1 genetic polymorphism with embryo implantation failure, *Braz. J. Med. Biol. Res.* 40 (2007) 793–797.
- [118] J. Zhang, Z. Chen, G.N. Smith, B.A. Croy, Natural killer cell-triggered vascular transformation: maternal care before birth? *Cell. Mol. Immunol.* 8 (2011) 1–11.
- [119] T. Steck, R. Giess, M.W. Suetterlin, M. Bolland, S. Wiest, U.G. Poehls, J. Dietl, Leukaemia inhibitory factor (LIF) gene mutations in women with unexplained infertility and recurrent failure of implantation after IVF and embryo transfer, *Eur. J. Obstet. Gynecol. Reprod. Biol.* 112 (2004) 69–73.
- [120] E. Hambartsumian, Endometrial leukemia inhibitory factor (LIF) as a possible cause of unexplained infertility and multiple failures of implantation, *Am. J. Reprod. Immunol.* 39 (1998) 137–143.
- [121] C. Simon, A. Frances, G. Piquette, M. Hendrickson, A. Milki, M.L. Polan, Interleukin-1 system in the materno-trophoblast unit in human implantation: immunohistochemical evidence for autocrine/paracrine function, *J. Clin. Endocrinol. Metab.* 78 (1994) 847–854.
- [122] C. Simon, A. Mercader, M.J. Gimeno, A. Pellicer, The interleukin-1 system and human implantation, *Am. J. Reprod. Immunol.* 37 (1997) 64–72.
- [123] K.J. Lim, O.A. Odukoya, R.A. Ajjan, T.C. Li, A.P. Weetman, I.D. Cooke, The role of T-helper cytokines in human reproduction, *Fertil. Steril.* 73 (2000) 136–142.
- [124] S. Linjawi, T.C. Li, E.M. Tuckerman, A.I. Blakemore, S.M. Laird, Expression of interleukin-11 receptor alpha and interleukin-11 protein in the endometrium of normal fertile women and women with recurrent miscarriage, *J. Reprod. Immunol.* 64 (2004) 145–155.
- [125] F. Feroze-Zaidi, L. Fusi, M. Takano, J. Higham, M.S. Salker, T. Goto, S. Edassery, K. Klingel, K.M. Boini, M. Palmada, R. Kamps, P.G. Groothuis, E.W. Lam, S.K. Smith, F. Lang, A.M. Sharkey, J.J. Brosens, Role and regulation of the serum- and glucocorticoid-regulated kinase 1 in fertile and infertile human endometrium, *Endocrinology* 148 (2007) 5020–5029.
- [126] M.S. Salker, M. Christian, J.H. Steel, J. Nautiyal, S. Lavery, G. Trew, Z. Webster, M. Al-Sabbagh, G. Puchchakayala, M. Foller, C. Landles, A.M. Sharkey, S. Quenby, J.D. Aplin, L. Regan, F. Lang, J.J. Brosens, Deregulation of the serum- and glucocorticoid-inducible kinase SGK1 in the endometrium causes reproductive failure, *Nat. Med.* 17 (2011) 1509–1513.
- [127] A. Santi, R.S. Felsler, M.D. Mueller, D.M. Wunder, B. McKinnon, N.A. Bersinger, Increased endometrial placenta growth factor (PLGF) gene expression in women with successful implantation, *Fertil. Steril.* 96 (2011) 663–668.
- [128] M.H. van der Gaast, K. Beier-Hellwig, B.C. Fauser, H.M. Beier, N.S. Macklon, Endometrial secretion aspiration prior to embryo transfer does not reduce implantation rates, *Reprod. Biomed. Online* 7 (2003) 105–109.
- [129] J.G. Scotchie, M.A. Fritz, M. Mocanu, B.A. Lessey, S.L. Young, Proteomic analysis of the luteal endometrial secretome, *Reprod. Sci.* 16 (2009) 883–893.
- [130] N.J. Hannan, A.N. Stephens, A. Rainczuk, C. Hincks, L.J. Rombauts, L.A. Salamonsen, 2D-DiGE analysis of the human endometrial secretome reveals differences between receptive and nonreceptive states in fertile and infertile women, *J. Proteome Res.* 9 (2010) 6256–6264.
- [131] A. Barash, N. Dekel, S. Fieldust, I. Segal, E. Schechtman, I. Granot, Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing in vitro fertilization, *Fertil. Steril.* 79 (2003) 1317–1322.
- [132] L. Zhou, R. Li, R. Wang, H.X. Huang, K. Zhong, Local injury to the endometrium in controlled ovarian hyperstimulation cycles improves implantation rates, *Fertil. Steril.* 89 (2008) 1166–1176.
- [133] M.A. Karimzadeh, R.M. Ayazi, N. Tabibnejad, Endometrial local injury improves the pregnancy rate among recurrent implantation failure patients undergoing in vitro fertilisation/intra cytoplasmic sperm injection: a randomised clinical trial, *Aust. N. Z. J. Obstet. Gynaecol.* 49 (2009) 677–680.
- [134] R. Li, G. Hao, Local injury to the endometrium: its effect on implantation, *Curr. Opin. Obstet. Gynecol.* 21 (2009) 236–239.
- [135] G. Teklenburg, M. Salker, M. Molokhia, S. Lavery, G. Trew, T. Aojanpong, H.J. Mardon, A.U. Lokugamage, R. Rai, C. Landles, B.A. Roelen, S. Quenby, E.W. Kuijk, A. Kavelaars, C.J. Heijnen, L. Regan, J.J. Brosens, N.S. Macklon, Natural selection of human embryos: decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation, *PLoS One* 5 (2010) e10258-.
- [136] S. Quenby, G. Vince, R. Farquharson, J. Aplin, Recurrent miscarriage: a defect in nature's quality control? *Hum. Reprod.* 17 (2002) 1959–1963.