Reproductive BioMedicine Online (2013) 27, 453–460

www.sciencedirect.com
www.rbmonline.com

SYMPOSIUM: IMPLANTATION
REVIEW

The science of implantation emerges blinking into the light

Andrew M Sharkey [a,*], Nick S Macklon [b]

[a] *Department of Pathology and Centre for Trophoblast Research, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK;* [b] *Human Development and Health, Faculty of Medicine, University of Southampton, Princess Anne Hospital, Southampton SO16 YA, UK*
[*] Corresponding author. *E-mail address:* as168@cam.ac.uk (AM Sharkey).

Andrew Sharkey's research uses transgenic mice, primate and human models to study the molecular basis of endometrial receptivity and early implantation failure. Novel approaches have been developed for the molecular analysis of embryo/endometrial interactions, including gene therapy to transfect uterine epithelium *in vivo* for functional studies of gene action. The aim is to develop novel contraceptives and to improve diagnosis and treatment of infertility. A second major area of work involves a collaboration with Professor Ashley Moffett studying interactions between fetal trophoblast and uterine NK cells at the maternofetal interface during early pregnancy.

Abstract Although embryo implantation is essential for human survival, it remains an enigmatic biological phenomenon. Following fertilization, the resulting blastocyst must signal its presence to the mother, attach to the luminal epithelium of the endometrium and embed into the decidualising stroma. Failure to do so results in infertility, which affects around 9% of women. Subsequent placental development requires remodelling of maternal blood vessels by trophoblast cells from the placenta, that invade deep into the decidua. Failure in these very early stages can compromise fetal development, resulting in diseases of pregnancy such as intrauterine growth restriction or pre-eclampsia which can also impact on health in adulthood. Abnormal implantation therefore constitutes a significant disease burden in humans. Although we have known for many years that successful implantation requires an embryo that is competent to implant and an endometrium that is receptive, the molecular basis of these processes remains poorly understood. Our inability to identify implantation-competent embryos or to diagnose/treat the non-receptive endometrium therefore limits our ability to intervene through assisted reproduction techniques. This Implantation Symposium aims to review recent exciting developments in our understanding of the biology of early implantation and to highlight the rapid progress being made to translate these into improved diagnosis and treatment.

KEYWORDS: embryo, endometrium, implantation, infertility, receptivity

Introduction

Implantation is the multistep process by which the free-floating blastocyst attaches to the endometrium, invades through the epithelium and into the stroma beneath and begins to establish the placenta. Successful implantation requires three key factors: an embryo competent to implant, a receptive endometrium and a successful

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2013.08.005

paracrine dialogue between the two. This 'conversation' is mediated by local factors, including hormones, cytokines, prostaglandins and adhesion molecules, and results in local changes in the epithelium and the embryo which permit attachment. However it has long been clear that implantation in humans is relatively inefficient compared with other species. Estimates, from both natural cycles and in-vitro fertilization-embryo transfer (IVF-ET) techniques, suggest implantation rates per embryo of around 25% in human populations of normal fertility (Macklon et al., 2002). This is substantially lower than the estimates of >70% in primates and rodents and even higher rates in domestic animals. Human couples also experience high rates of infertility, defined as the inability to conceive after a year of unprotected intercourse. Studies suggest that 1 in 6 couples experience infertility and no specific cause is identified in up to one-third of these. This points to a 'diagnostic gap' which reflects our ignorance of the factors in the endometrium and embryo that contribute to implantation failure.

The development of assisted reproduction techniques, such as IVF by Robert Edwards and Patrick Steptoe in 1978, has provided potential treatments for many infertile couples and resulted in the recent milestone of over 5 million babies born as a result of the use of these techniques. However there are still many women who fail to achieve pregnancy despite transfer of multiple embryos over several cycles (Malizia et al., 2013). While poor embryo quality undoubtedly contributes to this recurrent implantation failure (RIF), embryo donation studies suggest that a failure to achieve endometrial receptivity can also be a major factor. Many women with benign gynecological diseases that are associated with subfertility such as endometriosis, hydrosalpinx, and fibroids show abnormal endometrial responses to progesterone. Upon surgical treatment, normal endometrial development is usually restored and correlates with improved implantation (Cakmak and Taylor, 2011; Lessey, 2011). These observations strongly support the idea that failure to achieve a receptive endometrium contributes to infertility in many women. The widespread use of assisted reproduction techniques has thus enabled us to learn a great deal about the biology of human implantation that would otherwise have been ethically and practically difficult to achieve. However, the inappropriate use of IVF techniques has also introduced new problems. Because of the expense and emotional burdens associated with assisted reproduction, couples frequently opt to replace multiple embryos to overcome the 'low' success rates. Recognition of the impact of this practice on the risk of premature delivery and the associated long term consequences for health has lead to the adoption of mandatory single embryo transfer (SET) in many countries (Sunderam et al., 2012). The effort to maximize implantation rates for single transferred embryos has refocused attention on some of the fundamental practical questions associated with implantation:

1. What is the molecular basis of endometrial receptivity and can we identify biomarkers of the receptive endometrium?
2. Why do some women apparently fail to develop a receptive endometrium, and can improved diagnosis of the receptive state be used to enhance clinical decisions about whether to transfer embryos in any one treatment cycle?
3. Why do human pre-implantation embryos exhibit such a high rate of abnormality and how can embryos with the highest implantation potential be selected for replacement?

Implantation is a multistep process

Human embryo development and implantation are difficult to study *in vivo* for obvious ethical and practical reasons. Much of what we believe to occur is extrapolated from animal models coupled with observations of human embryo development *in vitro* following IVF. The establishment of systems in which blastocysts have been co-cultured with polarized endometrial epithelial cells has permitted study of embryo/epithelial attachment events (Bentin-Ley and Lopata, 2000). These in-vitro observations are supported by a limited number of histological specimens of early implantation sites obtained *in situ* (Hertig et al., 1956; Lindenberg, 1991). Following fertilization in the fallopian tube, the embryo initiates division and enters the uterine cavity at the morula stage approximately 72–96 h after fertilization. There it develops into a blastocyst and then hatches from the zona pellucida. During the pre-attachment phase a dialogue between the endometrium and the embryo is mediated by soluble factors. This results in apposition of the hatched blastocyst in the correct orientation to the epithelium (**Figure 1**). Local changes in adhesion molecules culminate in firm attachment of the embryo to the endometrium during which trophectoderm cells invade between the luminal epithelial cells. They penetrate the basement membrane and invade into the underlying stroma where they stimulate decidualization of the stromal cells.

Evidence that paracrine signals from the embryo prior to attachment may be involved in developing a fully receptive endometrium comes from both animal and human studies (Wang and Dey, 2006). Although receptivity depends primarily on sequential exposure of the endometrium to oestrogen followed by progesterone, changes in endometrial gene expression *in vivo* are seen in the presence of an embryo that are not seen in non-fecund cycles despite an apparently identical steroid profile (Duncan et al., 2011; Van Vaerenbergh et al., 2010). Human chorionic gonadotrophin (HCG) secreted by the pre-implantation embryo has a well-known role in maintaining pregnancy by preventing involution of the corpus luteum. In addition, it has also been shown to be one mediator of the local dialogue between the pre-implantation embryo and endometrium. Infusion of HCG into the uterine cavity in studies *in vivo*, and *in vitro*, have shown that this hormone regulates multiple genes in the endometrium (Horne et al., 2009; Licht et al., 2007; Sherwin et al., 2007). Interestingly these responses to HCG are altered in a baboon model of endometriosis (Sherwin et al., 2010). This result suggests that altered responses to HCG at the earliest stages of pregnancy may contribute to the reduced implantation rates associated with this condition.

These global pre-implantation responses should be distinguished from the local responses induced by the embryo at the implantation site itself following attachment. These arise as a result of paracrine signalling by soluble factors such as IGF1 from the embryo as well as due to direct

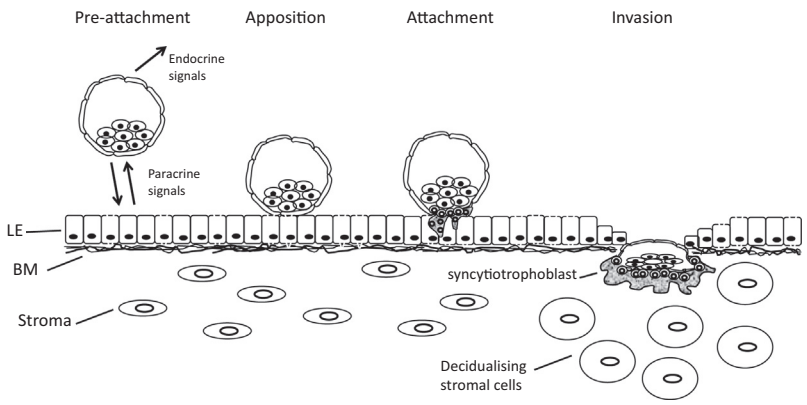

Figure 1 Schematic representation of the early stages of human implantation. The pre-implantation embryo signals its presence to the mother by both endocrine mediators, such as human chorionic gonadotrophin, and paracrine growth factors, which act locally on the endometrium to facilitate attachment. Following attachment the embryo penetrates the luminal epithelium (LE), breaches the basement membrane (BM) and invades into the underlying stromal cells. These begin to decidualise in earnest, although in humans some pre-decidual change is evident prior to embryo attachment. A detailed description of early human implantation stages by Professor Allen Enders, together with photomicrographs, is available to view from the website of the Centre for Trophoblast Research (http://www.trophoblast.cam.ac.uk/info/enders.shtml).

cell–cell interactions (Simón et al., 1997). These signals trigger local responses in the epithelium to promote attachment. They also promote the process of stromal cell decidualization that in humans begins shortly after ovulation in response to progesterone (Gellersen et al., 2007). Stromal cells transform into a highly secretory pseudoepithelial cell-type with a unique pericellular extracellular matrix. A key approach in studying such interactions has been the development of in-vitro culture systems in which embryos can be co-cultured with epithelial and/or stromal components. This set-up has permitted the dissection of specific local responses, the identification of the soluble embryo-derived factors responsible and, crucially, the functional role the factors play in embryo attachment. These interactions are complex with multiple cells types growing in three dimensions. The development of novel in-vitro implantation models which can more properly mimic the in-vivo process will be crucial if we are to fully understand how the interactions between embryo, epithelium and stroma contribute to successful implantation. These models are reviewed by Weimar and colleagues in this Symposium (Weimar et al., 2013).

Abnormal responses to the embryo may underlie implantation failure in some patients

Such in-vitro models have been used to compare responses to the embryo of primary cells from women of normal fertility with those from women with recurrent pregnancy loss (RPL). This approach has led to significant recent breakthroughs in our understanding of abnormal implantation. When decidualised stromal cells (DSC) were co-cultured with embryos that subsequently arrested, the secretion of cytokines, including IL-1beta, -6, -10, -17, and -18, was strongly down-regulated (Teklenburg et al., 2010). This outcome was in marked contrast to co-culture with normally developing embryos which stimulated little change. One interpretation is that DSC may act as biosensors of embryo quality, acting to recognize and prevent implantation of poor quality embryos. Although speculative, the model is supported by the finding that DSC from women with RPL (defined as 3 or more consecutive miscarriages) do not mount a response to abnormal embryos in the same way as DSC from fertile controls (Weimar et al., 2012). The idea that stromal cell decidualization is not simply a passive response to restrict embryo invasion but may also be part of an active embryo-selection process is reviewed by Lucas and colleagues in this Symposium (Lucas et al., 2013). This idea is also important because the different responses to abnormal embryos shown by DSC from fertile and RPL patients persist after passage of stromal cells *in vitro*. This observation suggests the novel idea that epigenetic mechanisms may underlie altered decidual responses in different patient groups that then result in implantation failure.

Effects of the periconceptional environment on the embryo

Embryo signalling to the endometrium is only one side of the coin. There is now considerable interest in the role of the endometrium and peri-conceptional environment on the subsequent development of the embryo. As the number of children born following assisted reproduction treatment increases, it is now important to examine their development through childhood and into adults. Animal studies suggest that even brief periods of embryo culture are associated with phenotypic changes in the offspring derived from such embryos compared with in-vivo derived counterparts. These include altered metabolism and increased blood pressure (Watkins et al., 2007). Comparison of the effects on mouse embryos of different commercially available culture media and protocols used for human IVF has revealed significant effects on embryonic development and gene expression, litter size and birth weight (Nelissen et al., 2013; Schwarzer et al., 2012). These findings raise important questions. The pre-implantation period is one of substantial epigenetic modification of the embryonic genome (Senner, 2011; Torres-Padilla, 2013). Changes in methylation or histone

modifications of selected genes have been reported in both animal and human embryos as a result of embryo culture (Mann and Denomme, 2013). Alterations in the cell lineages established in the pre-implantation embryo clearly have the potential to alter later fetal development. These differences may not be limited to the effects of in-vitro culture or other assisted reproduction techniques. Pre-conception maternal dietary supplementation in women has been reported to be associated with altered methylation at selected loci in female (but not male) children at nine months of age, although the functional consequences of this are not clear (Cooper et al., 2012). This finding indicates that factors such as maternal nutrient status at implantation may also influence fetal development, as has already been shown later in gestation (Fleming et al., 2012). The potential role of epigenetic effects on the embryo of the peri-conceptional environment including the use of assisted reproduction techniques is reviewed by Lucas (2013) in this Symposium. This rapidly developing area of research potentially has major implications for clinical practice.

Endometrial receptivity and implantation

Classical embryo transfer studies in animals and humans have shown that the endometrium only permits embryo implantation for a limited period. In mice this receptive period lasts for 24 h between days 4 and 5 after ovulation, after which time the endometrium again becomes refractory (Psychoyos, 1986). In humans this 'window of implantation' is less well-defined but extends from approximately day 5 to day 10 after the LH surge (Navot et al., 1991). For implantation to succeed, the endometrium needs to transition from the pre-receptive state to receptivity, after which it again becomes non-receptive (post-receptive). In all species the development of receptivity depends on the sequential exposure of the endometrium to oestrogen followed by progesterone. These hormones act via an array of nuclear receptors to regulate the transcription of hundreds of genes and to orchestrate the complex cellular and morphological changes that underlie the functional transition from pre-receptive to receptive state (Ruiz-Alonso et al., 2012).

A key aim of many recent studies has therefore been to define the mechanisms by which these steroids bring the endometrium to a receptive state. Particular attention has focused on the identification of morphological and molecular markers that identify endometrium that is functionally receptive. Two main strategies have been adopted:

1. Identifying RNA transcripts or proteins whose up- or down-regulation correlates with the transition from pre-receptive to receptive state (i.e. biomarkers of receptivity).
2. Comparing gene expression during the window of receptivity between fertile women and various 'subfertile' groups.

Over the last 25 years this huge effort has resulted in the identification of hundreds of genes where expression correlates with the window of implantation (Haouzi et al., 2012). Using techniques such as gene targeting, many of these genes have also been shown to play an important functional role (Wang and Dey, 2006). However the parallel effort in humans has been largely disappointing. Although it has been possible to identify many transcripts and proteins for which expression correlates with the receptive period, the substantial variation between women means that it has not proved possible to develop biomarkers that can reliably identify endometrium that is functionally receptive. An excellent example of this is the pioneering work on expression of the integrin family in the endometrium by Lessey et al. (1995), who showed that the expression of ITGB3 protein is upregulated in luminal and glandular epithelium at around LH+5 (Lessey et al., 1995). This time is when the endometrium is expected to become receptive. They also noted a frequent failure to upregulate ITBG3 at the appropriate time in women with unexplained infertility or endometriosis (Lessey, 2002). Although this failure or delay does also occur in some women with normal fertility, it was much more frequent in the subfertile groups. These findings have been echoed in many subsequent studies. Although individual markers can be identified where expression correlates with the implantation window, the variation seen, even within the normal fertile group, precludes their use as markers of the receptive state for any one individual patient.

To overcome this problem many groups have turned to high throughput technologies, such as gene expression microarrays, to determine the complete expression profile of pre-receptive and receptive endometria. They have also examined how this expression is perturbed in patient groups with presumed failure to achieve receptivity. There are considerable disparities among studies in the number of genes reported as altered during the transition to receptivity. Much of this may be due to differences in patient methods and to technical issues such as differences between array platforms, statistical methods used and sampling and RNA processing protocols (reviewed by Haouzi et al., 2012). In general this approach has confirmed the earlier picture of considerable variation among fertile women and even in the same woman from one cycle to the next (Ruiz-Alonso et al., 2013). More recently a consensus has emerged about how to make progress by designing algorithms to combine expression array data for multiple genes. Using a statistical meta-analysis of existing array data, Díaz-Gimeno et al. (2011) have derived the Endometrial Receptivity Array (ERA). This array comprises 238 transcripts that alter between the pre-receptive and receptive phases. In preliminary trials the ERA was able to classify correctly endometrium from normal and subfertile women (Díaz-Gimeno et al., 2011). If this approach is to make the transition from research study to clinical tool it will need to be shown to contribute usefully to clinical decision-making such as the timing of embryo replacement in women (Ruiz-Alonso et al., 2013).

It is worth reflecting on why it has been so difficult to define markers of receptivity, even when using techniques that are able to scan the entire genome. Progesterone triggers widespread changes in the endometrium, including differentiation and secretion by epithelial cells, stromal decidualization, vascular remodeling angiogenesis and the rapid influx of leukocytes. These changes are rapid and do not affect the basalis and functionalis of the endometrium equally. It is not surprising that random biopsies of

endometrium with different proportions of stromal, epithelial and leukocyte compartments have proved difficult to compare between patients. Despite this difficulty, the microarray approaches have enormously enhanced our understanding of how progesterone acts to co-ordinate the onset of receptivity. These recent advances are reviewed by Young in this Symposium (Young, 2013).

This approach has uncovered new details about how an abnormal response to progesterone may underlie infertility in some patients. It has also revealed the heterogeneity that can manifest as implantation failure among patient groups. For example in women with endometriosis there is evidence of selective failure of some responses to progesterone (Burney et al., 2007). This approach promises an era of more personalized treatment for patients based on an understanding of their specific underlying progesterone responses. Tangible clinical benefits have already resulted because microarray analysis has confirmed that ovarian stimulation causes changes to the receptive-phase endometrium that may be detrimental to implantation (Horcajadas et al., 2008). This realization has prompted a rethink about stimulation protocols that more naturally mimic the natural cycle, and/or a reconsideration of embryo freezing with replacement in subsequent cycles (Evans et al., 2012a). Secondly, in considering the early stages of implantation most scientists have ignored the fact that the luminal epithelium (LE) to which the blastocyst adheres and signals has a different phenotype to that of the glandular epithelium. More focused studies of specific compartments such as the LE are clearly warranted (Evans et al., 2012b). These studies are likely to identify further genes such as *SGK1*, one of the few examples to emerge from the gene array studies for which there is good evidence for a functional role in human implantation (Salker et al., 2011).

Novel approaches to biomarkers of receptivity

An alternative to the use of RNA microarrays to identify biomarkers of receptivity is the use of large-scale proteomic techniques. Two main approaches have been employed: analysis of whole endometrial tissue biopsies, and analysis of proteins secreted by the endometrium and present in uterine fluid. The latter presents a number of advantages in terms of ready access to samples, and the ability to use well-established protein assay systems such as 2D-gel electrophoresis and ELISA for protein discovery and subsequent quantitation (Boomsma et al., 2009; Cheong et al., 2013; Salamonsen et al., 2013). Uterine fluid is also largely derived from secretions of the luminal and glandular epithelia, which should closely reflect the receptive state of the principal cells with which the pre-implantation embryo interacts. The approach also offers the opportunity for rapid assessment of endometrial receptivity with minimal disturbance shortly before making a decision about embryo replacement. This rapidly developing field is reviewed in this Symposium by Edgell et al. (2013).

A novel component of the endometrium, the study of which is at an early phase, is microRNA (miRNA). These short non-protein coding RNAs are potential epigenetic regulators of endometrial gene expression and function. They are generated in the cytoplasm by the action of the Dicer enzyme from pre-miRNAs following export from the nucleus. They can act to silence or enhance RNA translation by binding to the 3′ untranslated region of selected mRNAs as well as by regulating mRNA stability (Filipowicz et al., 2008). miRNAs are expressed in the endometrium in a cell-specific manner and are steroid regulated, showing variation through the cycle — just as do their mRNA counterparts (Kuokkanen et al., 2010; Pan et al., 2007). More significantly, miRNAs show specific changes in expression during the transition from pre-receptive to receptive state in both mice and humans uteri (Altmäe et al., 2013; Chakrabarty et al., 2007). Finally comparison of receptive phase endometria among various subfertile groups, including those with RIF and endometriosis, has identified miRNAs that differ when compared with fertile controls (Revel et al., 2011). The potential to identify and use miRNAs as receptive-phase biomarkers is assessed in this Symposium by Hull and Nisenblat (2013). However, as with candidate 'receptivity' mRNA species, these markers will require validation in other cohorts using the same rigorous criteria to establish whether miRNAs have potential as biomarkers of receptivity.

Practical therapies to improve implantation outcomes

A key purpose of implantation research must be the translation into improved diagnosis and clinical practice, particularly for assisted reproduction techniques. The need for this is highlighted by the Symposium article by Fatemi and Popovic-Todorovic (2013) who review current clinical approaches to optimizing endometrial receptivity. Large randomised controlled trials (RCT) have shown that several widely advocated treatments for problems such as RIF and miscarriage offer no significant benefit for the majority of patients. These treatments include aspirin, low molecular weight heparin and corticosteroids. This outcome probably reflects heterogeneity in the underlying pathogenesis of such conditions and it remains to be seen whether these treatments could eventually be better targeted. Targeting will require development of screening systems to identify particular subgroups of patients who might benefit. These studies demonstrate the value of well-designed RCT for new potential assisted reproduction treatments, and their use has been a welcome step. However, clinical practice in this area is shown to remain largely empirical, although a number of promising approaches are emerging.

In recent years the principal focus of improving clinical outcomes from IVF has been the optimization of embryo selection for transfer. The imperative for this has derived primarily from the need to reduce the number of embryos transferred while maintaining improved pregnancy rates. It has long been apparent that human embryos exhibit much higher rates of chromosomal abnormalities than embryos of other species. Techniques that can screen the entire genome of single blastomeres have now revealed an even greater degree of abnormality than previously suspected in human embryos derived from IVF. Multiple microdeletions, duplications and rearrangements are present in most blastomeres. This finding has profound implications for techniques such as preimplantation genetic diagnosis as well as for our understanding of quality control in human

embryos (Ledbetter, 2009). In the Symposium paper by Montag et al. (2013) emerging technologies in the field of morphokinetics and the analysis of embryo-conditioned medium are reviewed. The use of continuous morphological assessment of embryo development to identify embryos with the highest potential for successful implantation is one of the most exciting developments in recent years. When coupled with the ability to vitrify embryos it could revolutionise the practice of assisted reproduction treatment. A single stimulation cycle could result in multiple embryos, which are frozen and replaced sequentially in subsequent cycles, starting with those of highest potential. Further refinements in the assessment of early embryo development may be anticipated, as novel embryo–endometrial interaction models (addressed in the Symposium article by Weimar et al., 2013) promise a more functional interrogation of embryo quality.

Conclusions

Further progress in the diagnosis and treatment of infertility in humans will be greatly hampered until we understand the molecular mechanisms underlying the three questions posed at the start of this review. Although these questions were identified in the early years of IVF, progress has until recently been rather limited. The development of methods such as laser capture and whole genome microarray and proteome techniques has begun to revolutionise our understanding of the complex changes in RNA and protein expression associated with the onset of endometrial receptivity and decidualisation. Active development of biomarkers for defining endometrial receptivity at the molecular level, together with clinical trials to test their validity, is underway (Ruiz-Alonso et al., 2013). These studies have revealed new insights into how the endometrium differs between fertile women and those with different types of infertility. Application of high sensitivity single cell techniques to single blastomeres has also permitted us to glimpse the molecular basis underlying variations in embryo quality. Finally the use of high-resolution techniques for monitoring embryo morphology and cell division promises the potential to rigorously identify embryos with high implantation potential. This single breakthrough would revolutionize the clinical practice of much assisted reproduction treatment and reduce the emotional and financial burdens to patients.

We remain on the cusp of being able to translate years of basic research into assays that can make a real difference in the clinic, but this translation will require the rigorous use of and careful study design. Assisted reproduction treatment has long since graduated from a 'cottage industry' in which small-scale studies based on the practice of one or two clinics was the norm. These studies were frequently statistically underpowered and often yielded contradictory results (Walters, 2013). They were thus of limited value to clinicians and patients alike as they sought to decide on the best treatments for their specific circumstances. The scale of the use of assisted reproduction treatments use and their potential impact on human health are such that it is essential that the very promising basic science research now underway is translated into the clinic as rapidly as possible following rigorous and statistically robust clinical trials. This course of action would allow all those in the field to contribute to fulfilling the vision and ambition of Bob Edwards and Patrick Steptoe when they first began to work on human implantation.

Acknowledgements

AS was supported by funding from the Wellcome Trust (090108/Z/09/Z, 085992/Z/08/Z), and would also like to thank the Centre for Trophoblast Research, Cambridge for generous support. NSM was supported in part by the Netherlands Organisation for Scientific Research (NWO) and the National Institute for Health Research (NIHR) in the UK.

References

Altmäe, S., Martinez-Conejero, J.A., Esteban, F.J., Ruiz-Alonso, M., Stavreus-Evers, A., Horcajadas, J.A., Salumets, A., 2013. MicroRNAs miR-30b, miR-30d, and miR-494 regulate human endometrial receptivity. Reprod. Sci. 20, 308–317. http://dx.doi.org/10.1177/1933719112453507.

Bentin-Ley, U., Lopata, A., 2000. In vitro models of human blastocyst implantation. Baillieres Best Pract. Res. Clin. Obstet. Gynaecol. 14, 765–774.

Boomsma, C.M., Kavelaars, A., Eijkemans, M.J., Lentjes, E.G., Fauser, B.C., Heijnen, C.J., Macklon, N.S., 2009. Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. Hum. Reprod. 24, 1427–1435.

Burney, R.O., Talbi, S., Hamilton, A.E., Vo, K.C., Nyegaard, M., Nezhat, C.R., Lessey, B.A., Giudice, L.C., 2007. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. Endocrinology 148, 3814–3826.

Cakmak, H., Taylor, H.S., 2011. Implantation failure: molecular mechanisms and clinical treatment. Hum. Reprod. Update 17, 242–253. http://dx.doi.org/10.1093/humupd/dmq037.

Chakrabarty, A., Tranguch, S., Daikoku, T., Jensen, K., Furneaux, H., Dey, S.K., 2007. MicroRNA regulation of cyclooxygenase-2 during embryo implantation. Proc. Natl. Acad. Sci. U. S. A. 104, 15144–15149.

Cheong, Y., Boomsma, C., Heijnen, C., Macklon, N., 2013. Uterine secretomics: a window on the maternal-embryo interface. Fertil. Steril. 99, 093–99.

Cooper, W.N., Khulan, B., Owens, S., Elks, C.E., Seidel, V., Prentice, A.M., Belteki, G., Ong, K.K., Affara, N.A., Constancia, M., Dunger, D.B., 2012. DNA methylation profiling at imprinted loci after periconceptional micronutrient supplementation in humans: results of a pilot randomized controlled trial. FASEB J. 26, 1782–1790.

Díaz-Gimeno, P., Horcajadas, J.A., Martínez-Conejero, J.A., Esteban, F.J., Alamá, P., Pellicer, A., Simón, C.A., 2011. Genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. Fertil. Steril. 95, 50–60.

Duncan, W.C., Shaw, J.L., Burgess, S., McDonald, S.E., Critchley, H.O., Horne, A.W., 2011. Ectopic pregnancy as a model to identify endometrial genes and signaling pathways important in decidualization and regulated by local trophoblast. PLoS One 6, e23595. http://dx.doi.org/10.1371/journal.pone.0023595.

Edgell, T.A., Rombauts, L.J.F., Salamonsen, L.A., 2013. Assessing receptivity in the endometrium: the need for a rapid, non-invasive test. Reprod. Biomed. Online 27, 486–496.

Evans, J., Hannan, N.J., Hincks, C., Rombauts, L.J., Salamonsen, L.A., 2012a. Defective soil for a fertile seed? Altered endometrial development is detrimental to pregnancy success. PLoS One 7, e53098.

Evans, G.E., Martínez-Conejero, J.A., Phillipson, G.T., Simón, C., McNoe, L.A., Sykes, P.H., Horcajadas, J.A., Lam, E.Y., Print, C.G., Sin, I.L., Evans, J.J., 2012b. Gene and protein expression signature of endometrial glandular and stromal compartments during the window of implantation. Fertil. Steril. 97, 1365—1373.

Fatemi, H.M., Popovic-Todorovic, B., 2013. Implantation in ART: a look at endometrial receptivity. Reprod. Biomed. Online 27, 530—538.

Filipowicz, W., Bhattacharyya, S.N., Sonenberg, N., 2008. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat. Rev. Genet. 9, 102—114. http://dx.doi.org/10.1038/nrg2290.

Fleming, T.P., Velazquez, M.A., Eckert, J.J., Lucas, E.S., Watkins, A.J., 2012. Nutrition of females during the peri-conceptional period and effects on foetal programming and health of offspring. Anim. Reprod. Sci. 130, 193—197.

Gellersen, B., Brosens, I.A., Brosens, J.J., 2007. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. Sem. Reprod. Med. 25, 445—453.

Haouzi, D., Dechaud, H., Assou, S., De Vos, J., Hamamah, S., 2012. Insights into human endometrial receptivity from transcriptomic and proteomic data. Reprod. Biomed. Online 24, 23—34.

Hertig, A.T., Rock, J., Adams, E.C., 1956. A description of 34 human ova within the first 17 days of development. Am. J. Anat. 98, 435—493.

Horcajadas, J.A., Mínguez, P., Dopazo, J., Esteban, F.J., Domínguez, F., Giudice, L.C., Pellicer, A., Simón, C., 2008. Controlled ovarian stimulation induces a functional genomic delay of the endometrium with potential clinical implications. J. Clin. Endocrinol. Metab. 93, 4500—4510.

Horne, A.W., Duncan, W.C., King, A.E., Burgess, S., Lourenco, P.C., Cornes, P., Ghazal, P., Williams, A.R., Udby, L., Critchley, H.O., 2009. Endometrial cysteine-rich secretory protein 3 is inhibited by human chorionic gonadotrophin, and is increased in the decidua of tubal ectopic pregnancy. Mol. Hum. Reprod. 15, 287—294. http://dx.doi.org/10.1093/molehr/gap019.

Hull, M.L., Nisenblat, V., 2013. Tissue and circulating microRNAs influence reproductive function in endometrial disease. 27, 515—529.

Kuokkanen, S., Chen, B., Ojalvo, L., Benard, L., Santoro, N., Pollard, J.W., 2010. Genomic profiling of microRNAs and messenger RNAs reveals hormonal regulation in microRNA expression in human endometrium. Biol. Reprod. 82, 791—801. http://dx.doi.org/10.1095/biolreprod.109.081059.

Ledbetter, D.H., 2009. Chaos in the embryo. Nat. Med. 15, 490—491. http://dx.doi.org/10.1038/nm0509-490.

Lessey, B.A., 2002. Implantation defects in infertile women with endometriosis. Ann. N. Y. Acad. Sci. 955, 265—280.

Lessey, B.A., 2011. Assessment of endometrial receptivity. Fertil. Steril. 96, 522—529. http://dx.doi.org/10.1016/j.fertnstert.2011.07.1095.

Lessey, B.A., Castelbaum, A.J., Sawin, S.W., Sun, J., 1995. Integrins as markers of uterine receptivity in women with primary unexplained infertility. Fertil. Steril. 63, 535—542.

Licht, P., Fluhr, H., Neuwinger, J., Wallwiener, D., Wildt, L., 2007. Is human chorionic gonadotropin directly involved in the regulation of human implantation? Mol. Cell. Endocrinol. 269, 85—92.

Lindenberg, S., 1991. Ultrastructure in human implantation: transmission and scanning electron microscopy. Baillieres Clin. Obstet. Gynaecol. 5, 1—14.

Lucas, E., 2013. Epigenetic effects on the embryo as a result of periconceptional environment and ART. Reprod. Biomed. Online 27, 477—485.

Lucas, E., Salker, M.S., Brosens, J.J., 2013. Uterine plasticity and reproductive fitness. Biomed. Online 27, 506—514.

Macklon, N.S., Geraedts, J.P., Fauser, B.C., 2002. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. Hum. Reprod. Update 8, 333—343.

Malizia, B.A., Dodge, L.E., Penzias, A.S., Hacker, M.R., 2013. The cumulative probability of liveborn multiples after in vitro fertilization: a cohort study of more than 10,000 women. Fertil. Steril. 99, 393—399. http://dx.doi.org/10.1016/j.fertnstert.2012.10.018.

Mann, M.R.W., Denomme, M.M., 2013. Maternal control of genomic imprint maintenance. Reprod. Biomed. Online (in press). http://dx.doi.org/10.1016/j.rbmo.2013.06.004.

Montag, M., Toth, B., Strowitzki, T., 2013. New approaches to embryo selection. Reprod. Biomed. Online 27, 539—546.

Navot, D., Bergh, P.A., Williams, M., Garrisi, G.J., Guzman, I., Sandler, B., Fox, J., Schreiner-Engel, P., Hofmann, G.E., Grunfeld, L., 1991. An insight into early reproductive processes through the in vivo model of ovum donation. J. Clin. Endocrinol. Metab. 72, 408—414.

Nelissen, E.C., Van Montfoort, A.P., Smits, L.J., Menheere, P.P., Evers, J.L., Coonen, E., Derhaag, J.G., Peeters, L.L., Coumans, A.B., Dumoulin, J.C., 2013. IVF culture medium affects human intrauterine growth as early as the second trimester of pregnancy. Hum. Reprod. 28, 2067—2074.

Pan, Q., Luo, X., Toloubeydokhti, T., Chegini, N., 2007. The expression profile of micro-RNA in endometrium and endometriosis and the influence of ovarian steroids on their expression. Mol. Hum. Reprod. 13, 797—806.

Psychoyos, A., 1986. Uterine receptivity for nidation. Ann. N. Y. Acad. Sci. 476, 36—42.

Revel, A., Achache, H., Stevens, J., Smith, Y., Reich, R., 2011. MicroRNAs are associated with human embryo implantation defects. Hum. Reprod. 26, 2830—2840. http://dx.doi.org/10.1093/humrep/der255.

Ruiz-Alonso, M., Blesa, D., Simón, C., 2012. The genomics of the human endometrium. Biochim. Biophys. Acta 1822, 1931—1942.

Ruiz-Alonso, M., Blesa, D., Díaz-Gimeno, P., Gómez, E., Fernández-Sánchez, M., Carranza, F., Carrera, J., Vilella, F., Pellicer, A., Simón, C., 2013. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil. Steril. http://dx.doi.org/10.1016/j.fertnstert.2013.05.004, pii:S0015-0282(13)00567-0.

Salamonsen, L.A., Edgell, T., Rombauts, L.J., Stephens, A.N., Robertson, D.M., Rainczuk, A., Nie, G., Hannan, N.J., 2013. Proteomics of the human endometrium and uterine fluid: a pathway to biomarker discovery. Fertil. Steril. 99, 1086—1092. http://dx.doi.org/10.1016/j.fertnstert.2012.09.013.

Salker, M.S., Christian, M., Steel, J.H., Nautiyal, J., Lavery, S., Trew, G., Webster, Z., Al-Sabbagh, M., Puchchakayala, G., Föller, M., Landles, C., Sharkey, A.M., Quenby, S., Aplin, J.D., Regan, L., Lang, F., Brosens, J.J., 2011. Deregulation of the serum- and glucocorticoid-inducible kinase SGK1 in the endometrium causes reproductive failure. Nat. Med. 17, 1509—1513. http://dx.doi.org/10.1038/nm.2498.

Schwarzer, C., Esteves, T.C., Arauzo-Bravo, M.J., Le Gac, S., Nordhoff, V., Schlatt, S., Boiani, M., 2012. ART culture conditions change the probability of mouse embryo gestation through defined cellular and molecular responses. Hum. Reprod. 27, 2627—2640.

Senner, C.E., 2011. The role of DNA methylation in mammalian development. Reprod. Biomed. Online 22, 529—535.

Sherwin, J.R., Sharkey, A.M., Cameo, P., Mavrogianis, P.M., Catalano, R.D., Edassery, S., Fazleabas, A.T., 2007. Identification of novel genes regulated by chorionic gonadotropin in baboon endometrium during the window of implantation. Endocrinology 148, 618—626.

Sherwin, J.R., Hastings, J.M., Jackson, K.S., Mavrogianis, P.A., Sharkey, A.M., Fazleabas, A.T., 2010. The endometrial response to chorionic gonadotropin is blunted in a baboon model of endometriosis. Endocrinology 151, 4982—4993.

Simón, C., Gimeno, M.J., Mercader, A., O'Connor, J.E., Remohí, J., Polan, M.L., Pellicer, A., 1997. Embryonic regulation of integrins beta 3, alpha 4, and alpha 1 in human endometrial epithelial cells in vitro. J. Clin. Endocrinol. Metab. 82, 2607–2616.

Sunderam, S., Kissin, D.M., Flowers, L., Anderson, J.E., Folger, S.G., Jamieson, D.J., Barfield, W.D.Centers for Disease Control and Prevention (CDC), 2012. Assisted reproductive technology surveillance — United States, 2009. MMWR Surveill. Summ. 61, 1–23.

Teklenburg, G., Salker, M., Molokhia, M., Lavery, S., Trew, G., Aojanepong, T., Mardon, H.J., Lokugamage, A.U., Rai, R., Landles, C., Roelen, B.A., Quenby, S., Kuijk, E.W., Kavelaars, A., Heijnen, C.J., Regan, L., Brosens, J.J., Macklon, N.S., 2010. Natural selection of human embryos: decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation. PLoS One 5, e10258.

Torres-Padilla, M.E., 2013. Generating different epigenotypes. Reprod. Biomed. Online (in press). http://dx.doi.org/10.1016/j.rbmo.2013.07.016.

Van Vaerenbergh, I., McIntire, R., Van Lommel, L., Devroey, P., Giudice, L., Bourgain, C., 2010. Gene expression during successful implantation in a natural cycle. Fertil. Steril. 93, 268.e15–268.e18. http://dx.doi.org/10.1016/j.fertnstert.2009.08.057.

Walters, E., 2013. A new title to an old song: some observations on the conduct of meta-analyses. Reprod. Biomed. Online 27, 562–567.

Wang, H., Dey, S.K., 2006. Roadmap to embryo implantation: clues from mouse models. Nat. Rev. Genet. 7, 185–199.

Watkins, A.J., Platt, D., Papenbrock, T., Wilkins, A., Eckert, J.J., Kwong, W.Y., Osmond, C., Hanson, M., Fleming, T.P., 2007. Mouse embryo culture induces changes in postnatal phenotype including raised systolic blood pressure. Proc. Natl. Acad. Sci. U. S. A. 104, 5449–5454.

Weimar, C.H.E., Post Uiterweer, E.D., Teklenburg, G., Heijnen, C.J., Macklon, N.S., 2013. In-vitro model systems for the study of human embryo–endometrial interactions. Reprod. Biomed. Online 27, 461–476.

Weimar, C.H., Kavelaars, A., Brosens, J.J., Gellersen, B., de Vreeden-Elbertse, J.M., Heijnen, C.J., Macklon, N.S., 2012. Endometrial stromal cells of women with recurrent miscarriage fail to discriminate between high- and low-quality human embryos. PLoS One 7, e41424. http://dx.doi.org/10.1371/journal.pone.0041424.

Young, S., 2013. Oestrogen and progesterone action on endometrium: a translational approach to understanding embryo receptivity. Reprod. Biomed. Online 27, 497–505.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 24 August 2013; accepted 27 August 2013.