

Review

Infertility and the Endometrium

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Abstract

Background: A couple's infertility can originate from the male and/or the female. In women, the uterus provides the site where the maternal-fetal interface is established and maintained. Final blastocyst development occurs within the uterine cavity, then the blastocyst must attach to and implant into the endometrium (the inner uterine surface), via its outermost trophoblastic cells. Beneath the epithelium, these differentiate into syncytial trophoblast and invasive trophoblast — the latter progress through the endometrium to invade the spiral arteries converting them to the flaccid blood sacs of the placenta. Therefore, the endometrium plays a critical role in establishment of pregnancy. **Objectives:** To critically examine current knowledge of endometrial preparation for blastocyst implantation and placental development at the cellular and molecular level and to evaluate measures to improve implantation success. **Mechanism:** Literature searching by leading experts in the field. **Findings:** A wealth of new knowledge resulting from 'omics' technologies and new functional models has greatly enhanced our knowledge, but this information is yet to be translated into enhanced outcomes. **Conclusions:** The endometrium remains the 'black box' of infertility. Extensive trials do not support current adjuvant therapies as being better than placebo while effectively timed testing for endometrial preparedness for implantation is still urgently needed.

Keywords: receptive endometrium; implantation; cellular composition; molecular analysis; embryo-maternal interactions; adjuvant therapies; testing for receptivity; infertility

1. Introduction

Successful establishment of pregnancy in women requires highly regulated implantation of a healthy embryo into the woman's endometrium (the lining of the uterus) and necessitates synchronised development of the two. The endometrium is highly dynamic throughout each menstrual cycle. The outer functional layer is shed at each menses, with the start of menses being taken as day 1 of the cycle, which is usually of 27–35 days duration. Under the influence of estradiol 17β (E) secreted from the developing ovarian follicles, the endometrium is fully restored following menses, predominantly from stem cells resident in the unshed basal layer [1]. Cellular proliferation and deposition of extracellular matrix (ECM) continue until mid-cycle, when the endometrium has achieved its full thickness. Following ovulation, and under the influence of progesterone (P) in the presence of E, all of the cell types (epithelial, stromal, vascular, immune) in the tissue undergo separate but coordinated differentiation, such that by the mid-secretory phase, the tissue attains a state of 'receptivity' for embryo implantation and the so-called "window of implantation (WOI)" is open. In a non-conception cycle, in the absence of the embryonic anti-luteolytic signal, human chorionic gonadotrophin (hCG), P and E levels fall rapidly, inducing a highly controlled inflammatory reaction in the tissue that leads to shedding of the tissue at menstruation and commencement of a new cycle [2]. This review will

summarise current knowledge including the events of implantation and placentation in women, including the cellular and molecular events at implantation, endometrial receptivity and the uterine microenvironment. Further, it will discuss important techniques recently available for the study of endometrial remodelling and embryo implantation along with the need for a reliable test for receptivity. Finally it will examine whether there is an evidence base for a range of adjuvant therapies currently used clinically.

2. Events of Implantation

The events of implantation and placentation vary widely between species; in particular they are very different in women than in most experimental animals, most commonly rats and mice in which the luminal epithelium is lost by entosis, and decidualization occurs only in the presence of an embryo, among other differences. In women, the first contact between the embryo and the endometrium, is between the epithelial outer cellular layer of the blastocyst, adjacent to the inner cell mass (the polar trophoblast), and the luminal epithelium of the endometrium. The latter normally forms a barricade guarding and protecting the endometrium from microbial infection and chemical injury. This barrier function must be weakened to allow embryo attachment and penetration. Since two such epithelial layers, (each of which are polarised with apical and basal surfaces), are naturally repulsive to one another, the polarity of



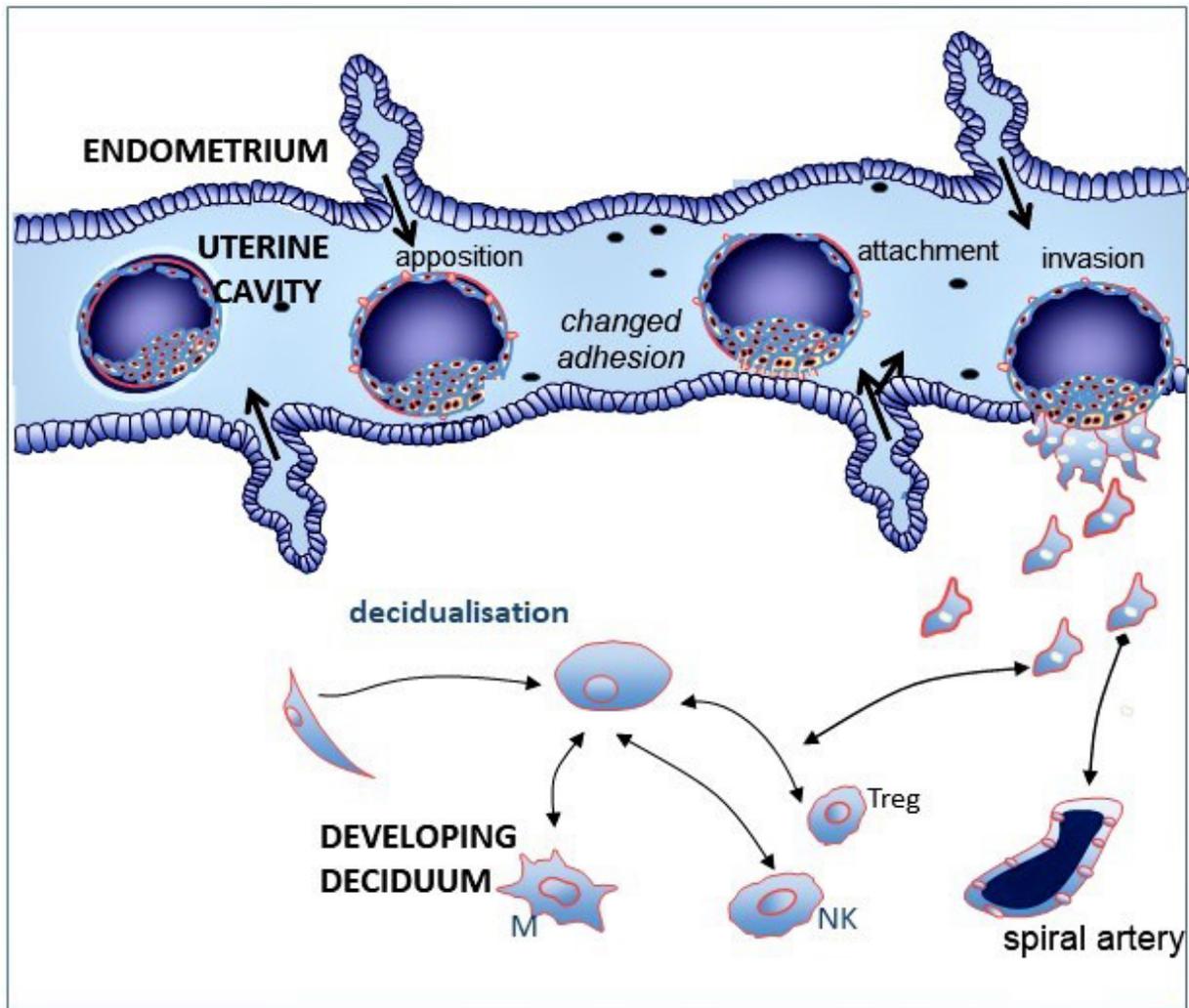


Fig. 1. Cellular events at implantation in women. The embryo enters the uterus as an unhatched blastocyst which has an inner cell mass, an outer layer of trophoblast and a blastocoel. After hatching it becomes apposed to, attaches to and invades the luminal epithelium and the trophoblastic cells differentiate to become syncytial trophoblast and invasive trophoblast. The latter continue through the decidual compartment to eventually invade and transform the spiral arteries. Differentiation of the epithelium, both luminal and glandular, precedes that of the stromal fibroblasts. The latter, stimulated by progesterone in concert with the invading macrophages (M), uterine NK cells (NK) and T regulatory cells (Treg) undergo the differentiation process of decidualisation to form the deciduum of pregnancy. All these processes involve differentiation of the various cell types, along with actions of their secreted products (including miRNA, proteins, lipids) and released extracellular vesicles. (modified from [14]).

the endometrial epithelium must change in preparation for implantation. Indeed, a decrease in their electronegativity and downregulation of polarity molecules has been demonstrated [3]. In this respect, the cells undergo a partial epithelial to mesenchymal transition which also relaxes the tight junctions that normally make the epithelial layer impenetrable [4]. Likewise, adhesion systems at the trophoblast-luminal epithelial interface are modified; these include the lectin-like protein L-selectin, integrins, the heparin-like EGF-like growth factor (HG-EGF) and its receptor Erb4 (reviewed: [5]) along with desmoplakin 1 [6], transmembrane MAML1, a component of the Notch pathway [7,8]

and podocalyxin [9]. Non-coding RNA, specifically microRNA [10] and long non-coding RNA [11] along with their intracellular processing machinery [12] alter endometrial epithelial cell capacity. Membrane-cytoskeletal interactions within the cell are also altered through molecular changes, which include cleavage of the scaffolding protein EBP-50 and the membrane protein dystroglycan, by the enzyme pro-protein convertase (PC) 5/6 [9]. Furthermore, the surfaces of both the implanting blastocyst and the endometrial epithelium are heavily glycosylated and interact in a highly controlled and specific manner. This pattern of glycosylation (the glyco-type) differs between species [13] pro-

viding an important mechanism that prevents interspecies breeding. Once the barrier presented by the luminal epithelium is relaxed, the trophoblastic cells can penetrate between the epithelial cells, then through the basal lamina to form a syncytium beneath the epithelial layer, from which invasive trophoblast cells arise. These traffic through the decidua, which exerts restraint, largely by the production of anti-proteases, until by the end of the first trimester they reach and invade the spiral arterioles which they transform into large conduits of low resistance. The vascular, invasive and syncytial trophoblast cells together form the fetal component of the placenta. This is summarised in Fig. 1 [14].

Also, in the early-mid secretory phase of each cycle, a differentiative process of the stromal cells commences in a small percentage of stromal fibroblasts around the spiral arterioles. This process is essentially a partial mesenchymal-epithelial transition. The fibroblasts acquire pavement-like morphology, each surrounded by a basal lamina and become highly secretory, releasing a plethora of regulatory molecules including chemokines which support leukocyte infiltration. This is known as decidualization and is driven and maintained by rising P levels. After initiation of decidualization, local paracrine factors create a slow 'wave' of decidualization that spreads throughout the endometrium so that in the receptive phase, decidualization features are widely spread in the stromal compartment. If pregnancy ensues in that cycle, the decidual cells both promote and restrain invasion of the fetal extravillous trophoblast cells and eventually form the maternal component of the placenta. The decidual cells, in conjunction with uNK, macrophages [15] and regulatory T cells (Treg) [16] all of which are present in high abundance at this time [17], also facilitate spiral artery remodelling [18], provide maternal immunotolerance to the fetal allograft [19], shield the conceptus from environmental stress signals [20] and 'sense' embryo quality to facilitate maternal rejection of developmentally incompetent embryos [21]. Quality control at implantation and beyond is also influenced by active engagement based on the adaptive immune response as recently emphasised by single cell sequencing [22] from which transcriptional regulation was seen as most dynamic in immune cells, particularly uNK and Treg, in the mid-secretory phase.

3. Endometrial Receptivity

Endometrial receptivity is achieved by actions of P in the presence of E, that cause essential phenotypic changes in all endometrial cells just before and during the mid-secretory phase of most menstrual cycles. Should receptivity not be attained, implantation cannot take place and any blastocyst present will be lost. Over recent decades many individual endometrial molecules of importance as paracrine mediators at implantation have been reported with actions on at least one endometrial cell type demonstrated; these include growth factors, chemokines, cytokines and

enzymes [23]. The recent ground-breaking, single cell transcriptomic atlas of the human endometrium [22], provided novel insights into menstrual cycle phase transitions, including identifying biomarkers that define the opening of the WOI, particular cell specific signatures and cell-cell interactions. These include differences between ciliated and unciliated epithelia: the latter are predominant in the luminal epithelium where attachment must occur although the function of ciliated epithelia is not known. The study also shows an abrupt opening of the WOI with transcriptional transition in unciliated epithelia, but a more gradual transition in stromal fibroblasts with interactions between these and uNK cells promoting decidualization. Indeed, development of the secretory endometrial phenotype is overall a continuum, with clear transitional changes differing temporally in the different cell types within the tissue. Importantly, decidualized fibroblasts from non-pregnant endometrium had a different transcriptional signature than those in early pregnancy; a unique observation that pregnancy itself may influence decidualization [24]. It is thus unlikely that there can be a consistent predictive 'receptive' state as defined by transcriptional regulation in non-pregnant tissues taken from a previous non-conception cycle and that the terms 'receptive endometrium' and 'WOI' should only be applied to studies that include (at least morphologically) well-characterised embryos implanting *in vivo* [25].

4. Uterine Microenvironment

The microenvironment of implantation is provided by the contents of uterine fluid (histotroph) within the uterine cavity. It is here that final pre-implantation development of the embryo occurs, supported by this rich molecular soup. The fluid includes the essential nutrients, (such as glucose, amino acids, lactate and fatty acids) along with soluble bioactive factors: these are secreted largely from the uterine glands which are essential for implantation, as demonstrated in animals engineered to lack glands [26,27]. Trophoblast also secretes soluble biomolecules into uterine fluid [28,29]. Furthermore extracellular vesicles (EV) are released into uterine fluid from both maternal and trophoblastic surfaces [30–32]. EV membranes protect their 'cargo' from enzymatic degradation and deliver these biomolecules, which include proteins, lipids and miRNA, to their specific target cells, thus changing their phenotype to promote implantation [33]. For most of the secreted mediators and for EVs, either specific receptors have been identified on the apposing surface or functional responses demonstrated [34,35]. Furthermore, secreted proteolytic enzymes such as proprotein convertase 5/6, can cleave cell surface proteins such as α -dystroglycan, that act as a barrier for embryo attachment [9]. Thus, an embryo-maternal dialogue is set up even before implantation and enables the process.

5. Effects of Stimulation or Hormone Replacement on Endometrial Receptivity

In IVF cycles the hormonal stimulation for multiple follicle development and hCG administration for ovulation, considerably impacts the endometrium. A major (immuno)-histological study identified hyperproliferation of blood vessels, advanced progression of stromal but not endometrial differentiation and changed leukocyte activation in stimulated cycles ([36] and reviewed in [37]). This data strongly supports the case for preferential frozen embryo transfer (FET) although an optimal hormonal support regime for FET is yet to be established [38] and controversy remains about the outcomes.

6. Effects of Elevated BMI

An elevated body mass index (BMI) is significantly associated with infertility and increased time to pregnancy [39]. Obese women take longer to become pregnant, and often have difficulty conceiving in a natural cycle [40]. Every unit of increase in BMI above 29 kg/m² reduces the likelihood of a natural pregnancy by 4% [41]. These poor outcomes are reflected in obese women undertaking assisted reproductive technology (ART) cycles with maternal obesity being associated with reduced implantation and clinical pregnancy compared with lean women [42], and reduction in live birth rate of 15% [43]. One potential explanation is that advanced glycation end products (AGEs), derivatives of fat and sugar-related molecules, are elevated 4-fold in uterine fluid of women with BMI >30 and these can detrimentally affect the developing embryo, particularly the ratio of trophoblastic to inner cell mass cells, endometrial epithelial adhesive and proliferative abilities and stromal cell decidualization. Thus, reduction in AGEs, particularly if managed by diet may enhance success rates of ART [44]. Pharmacological AGE inhibition is possible [45] but not ideal in the context of early pregnancy.

7. Effects of Ageing

The major effects of ageing on women's fertility are on oocyte development and ovulation. A human female is born with a fixed number of oocytes (around 400), cohorts of which develop during each reproductive cycle following puberty: hence only a low number remain as the woman approaches 40 years of age. Oocyte quality declines with age, and this can affect the development of the embryo after fertilization [46,47].

By contrast, the human endometrium is a highly regenerative tissue. Its functional layer is shed each month at menstruation during the reproductive years. The endometrium is restored (driven by E) during the proliferative phase of the next cycle, and then under the influence of E+P, differentiates to become receptive to embryo implantation by the early-mid secretory phase [2]. After menopause, when this hormonal stimulus ceases, the endometrium en-

ters a state of senescence, but can redevelop following treatment with appropriate exogenous hormones: this restored endometrium can support a pregnancy as shown clearly by the birth of children to aged women who have undergone endometrial stimulation and donor embryo transfer. The oldest woman reported as having a live birth with the help of IVF, was an Indian woman aged 74 (reported in the Times of India and later in the Washington News (USA Today 6 Sept, 2019)). However, even if high quality embryos are transferred into endometrium of appropriate thickness, infertility treatments may still fail in older women due to incorrect placentation and early embryo loss. Furthermore, the risk of pregnancy complications, including miscarriage, intrauterine growth retardation (IUGR), pre-eclampsia, placenta previa and stillbirth is elevated with advanced maternal age [48]. Interestingly, application of Horvath's epigenetic clock, which uses the methylation of 353 CpG sites in the human genome, to calculate an epigenetically based biological age of a tissue, has shown poor correlation with chronological age for the endometrium [49]. This is not unexpected due to the regular shedding and regeneration of this tissue.

Ageing in terms of reproductive capacity is generally considered to occur around the age of 40 years until the menopause is established, usually some 10 years later. Some studies indicate that the endometrial milieu may become more inflammatory as women age. Indeed, expression of the pro-inflammatory proteins interleukin (IL)17 receptor B, CXCL12 and CXCL14, as determined by immunohistochemistry, were significantly higher in women in their 40's than those in their 20's [50]. Furthermore, endometrial epithelial cells derived from hysterectomy samples, when stimulated with a viral mimic, responded by secretion of interferon γ 1, and this was significantly increased with increasing age of the tissue donor [51].

Endometrial regeneration after each menstruation is largely from endometrial stem/progenitor cells (eMSC), residing in the unshed endometrial basalis [1]. After menopause, the remaining thin atrophic endometrium is mainly luminal epithelium with a few inactive glands and stroma; these can be stimulated to regenerate full thickness endometrium following 6–8 weeks of estrogen (E) therapy. Indeed, the eMSC in postmenopausal women did not differ in terms of markers, from eMSC derived from their premenopausal counterparts [52]. These markers N-cadherin⁺ and SSEA⁺ along with ER⁺, are present in atrophic tissue in post-menopausal women and in those treated with E, with the same pattern as in pre-menopausal women [53]. The precursors of vascular stroma (SUSD2⁺eMSC) also survive E depletion and can subsequently proliferate in response to exogenous E via niche cells [52]. Thus, it appears that endometrial stem/progenitor cells lie dormant until E levels rise.

Gene profiling has determined that pre-menopausal and peri-menopausal eMSC exhibit similar transcriptomic

signatures, although in the same study, endometrial stromal fibroblasts from these two groups showed altered pathway activation [54]. Possibly of relevance here is that in a non-menstruating species, the laboratory mouse, there are major maternal-age associated problems of stromal decidualization, which interfere with subsequent placental development [55]. Most recently Devesa-Peiro *et al.* [56] unsupervised artificial intelligence methods to raw data from previously published transcriptomic studies and analysed normal endometrium from women of different ages with regular menstrual cycles using algorithms that defined age groups. They uncovered different transcriptomic profiles according to age, clearly grouping women into those <35 and those >35 years. Interestingly most of the upregulated transcripts were related to enhancement of ciliary processes, while down-regulated functions related to cell cycle arrest. While the data appear to be strong, the changes defined are difficult to interpret as entire endometrium was analysed, whereas endometrial stroma and epithelium individually have very different transcriptomic profiles [22,57]. Furthermore, just how these changes might be controlled, given that the functional endometrium is replaced monthly, clearly needs further investigation.

8. New Technologies for Study of Endometrial Remodelling and Embryo Implantation

Leaps in knowledge commonly arise from application of new tools or methodologies to unresolved issues. Emerging technologies are now providing new ways for examining endometrium and its readiness for implantation, overcoming some of the limitations of working with scarce human material. These include new genomic techniques, organoids and blastoids.

8.1 New Genomic Techniques

New technologies to profile cells at individual levels using a range of ‘omics (genomics, transcriptomics, epigenomics, proteomics) have become part of the tool box in biomedical laboratories over the past 2 decades. They enable profiling of tissues or cells at individual levels in an attempt to understand cellular and molecular changes that take place during transit from one state to another under the influence of both internal processes and external cues. These technologies have been applied widely to the endometrium (review: [25]), providing information on major pathways and divergences from these; such ‘big data’ are universally available on databases to researchers. Limitations include that the analyses are usually made on complex tissue containing multiple cell types, and that normal material from humans is very difficult to obtain compared with disease tissue that is commonly removed surgically.

A recent important development is single cell transcriptomics, (single cell RNA sequencing, [SC-RNA-seq]) which enables study of the heterogeneity of cells at an in-

dividual level (review: [58]). This requires cell isolation, lysis, amplification, cDNA generation, sequencing either of full-length transcripts or of partial sequences at either the 3’ or 5’ end of the transcript, along with complex platforms for analysis of the extensive data. Such sophistication requires broad collaborations between bioscientists and bioinformaticians. Recent applications of this technique to the normal cycling endometrium have considerably advanced our knowledge. Wang and team at Stanford University (USA), have defined the time-differences in differentiation between endometrial luminal epithelium and stromal fibroblasts, with phenotypic changes in the epithelium preceding those in the fibroblasts, in preparation for implantation [59]. Additionally, Garcia-Alonso, and a team based predominantly at Cambridge University (UK) have dissected the signalling pathways determining cell fate of the epithelial lineages in the luminal and glandular epithelium [60]. In a comparison of thin proliferative phase endometrium with that of normal thickness at single cell resolution, a subpopulation of stromal cells showed compromised cell cycle signalling in thin endometrium, with cellular senescence in both stroma and epithelium, collagen over-deposition around blood vessels and decreased numbers of macrophages and neutrophils [61]. Further application of single cell analysis, particularly if combined with spatial transcriptomics will provide invaluable knowledge of the requirements for successful implantation, with the proviso that the tissues for analysis are very carefully selected and documented.

8.2 Organoids

Organoids are tiny, self-organized three-dimensional tissue cultures that bear a resemblance to a patient’s own tissues and are derived from stem cells. Such cultures can be crafted to replicate much of the complexity of an organ, or to express selected aspects of it such as producing only certain types of cells. Recent efforts have established hormonally-responsive 3-dimensional uterine epithelial cell cultures now known as organoids. These organoids display long-term expandability and can be cryopreserved for subsequent studies [62–64]. Ciliogenesis in these can be driven by E [65], while organoids derived from women with endometrial disease have been shown to capture the clinical disease diversity [66]. Importantly, such organoids can be used for drug screening [66]. Single cell RNAseq analyses have provided gene expression atlases of the organoids [64] including that differentiation in E-stimulated organoids depends upon the epithelial cell types present [64] and that down regulation of WNT or NOTCH signalling increases differentiation efficiency along the secretory and ciliated pathways respectively [60]. Future developments include 3D-models containing both stromal, vascular, immune and epithelial cells. For example, a new ‘implantation-on-a-chip model that includes maternal preimplantation immune cells shows that these affect the

decidua of pregnancy [67]. Additional new models that enable study of the interactions between all cell types involved at implantation are eagerly awaited.

8.3 Embryo-Like Structures: Blastoids Enable Study of Implantation

Studying human implantation has been severely hindered by the lack of a model that truly represents the peri-implantation site. Many models have utilized various human trophoblast cell lines-developed into spheroids, placed them on monolayers of human endometrial epithelial cells (generally cell lines), treated appropriately with E and P; these have provided limited data (e.g., [68]). Regrettably, the trophoblast cell lines are derived from much later stages of trophoblast development than the trophectoderm of the pre-implantation blastocyst and the epithelial monolayer does not truly represent polarised luminal epithelium.

A very few studies have included human blastocysts as well as spheroids developed from trophoblast cells [69]: both formed syncytiotrophoblast upon interaction with the epithelium. However, human blastocysts are available only under strict ethical regulation and in very low numbers (review: [70]). More recently, embryo-like models derived from pluripotent stem cell (PSC) are emerging as experimental entities: names for these are still emerging — embryoids, synthetic entities with human embryo-like features (SHEEFs) and embryo-like structures (ELS). Most of these enable human development to be studied at early post-implantation [71].

Most recently, models of the pre-implantation blastocyst, now termed human blastoids, have been developed [72,73]. These resemble human blastocysts in their morphology, size, cell number, and their allocation of several cell lineages with different compositions. Their transcriptomic similarity to blastocysts has been confirmed by single-cell RNA seq. Furthermore, Kagawa and colleagues [74], have developed blastoids from primordial stem cells, by triple inhibition of the Hippo, TGF- β and ERK pathways. Under these conditions, blastoids form with more than 70% efficiency, and generate blastocyst-stage analogues of the three founding lineages (>97% trophectoderm, epiblast and primitive endoderm) according to the sequence and timing of blastocyst development. Blastoids spontaneously form the first axis, with the epiblast inducing the local maturation of the polar trophectoderm, thereby endowing blastoids with the capacity to directionally attach to E+P stimulated endometrial cells, as occurs during implantation. While these blastoids are suggested to be reliable and scalable models for investigating human implantation and embryonic development, the ethics and regulation of such material is currently under considerable scrutiny world-wide [71]. Furthermore, there is still no model to examine the expanding implantation sites, throughout the early days post-implantation when pregnancy loss is high.

9. Major Clinical Need: A Sensitive Non-Invasive Test for Endometrial Receptivity

Considerable effort using extensive transcriptomics and proteomics, has been applied to developing a test that can predict endometrial receptivity. A test now offered commercially to patients world-wide, the endometrial receptivity array (www.igenomix.com/our-services/era/) is based on a transcriptome analysis of 238 genes in LH+7 endometrial biopsies. However, these are P treated cycles with biopsies taken after 7 days of P treatment. Although this is called a ‘natural cycle’ there remain concerns about the inconsistency of biopsies, and variation from cycle to cycle. Furthermore, any effects of embryo-maternal dialogue are not included. The test requires an endometrial biopsy to provide tissue for testing, repeated medication cycles with testing to ‘stimulate’ receptivity and then a freeze-all cycle. The only large-scale global trial of women who had been tested by the ERA prior to IVF, suggested that its use may improve outcomes [75], while smaller trials reported variable results. Use of the ERA is not currently supported by many experts in the field [25,76]. Whether such a multifactorial test on total endometrial tissue will ever be effective is not clear. The variability of the implantation window, makes sampling time difficult to determine [25,77]. Indeed, a novel open access software (EndoTime) measuring just 6 genes (IL2RB, IGFBP1, CXCL14, DPP4, GPX3 and SLC15A2) in luteal phase endometrial biopsies offers potential for more accurately timing biopsies [78] but does not measure receptivity. A test based on differentiation markers within just one cell type in the tissue may prove more accurate [22,25]. However, endometrial biopsy is not without risk. Indeed, it is unlikely that sampling in the mid-secretory phase can effectively improve the chance of implantation and viable ongoing pregnancy. It has recently been proposed that the terms ‘receptivity’ and ‘WOI’ be confined to studies related to well-characterised embryos implanting *in vivo* [25] and we concur that it is time for such a change in terminology. Importantly, assays requiring less invasive sampling and which can accurately predict outcome prior to embryo transfer must form the basis of any universal endometrial test. This could be achieved by measurements of biomarkers (including EVs) in serum or uterine fluid sampled in time for results to be available before a decision on embryo transfer is made.

10. Adjuvant Therapies. Do they Help?

A plethora of adjuvant therapies, said to improve endometrial function, have been introduced into fertility clinics in recent years. Regrettably, evidence for their benefit is largely lacking and some therapies have even been shown as detrimental, yet the use of IVF-add-ons is mostly unregulated. They are used particularly by women with repeated implantation failure, who are becoming desperate to conceive. A number of add-ons make the claim that they

will improve endometrial receptivity and hence increase the probability of live birth. Recent reviews of the evidence supporting such adjuvants include [79–82]. Surely it is time that vulnerable couples are warned against expensive unproven treatments.

10.1 Immune Therapies

It has been suggested that the immune system may be dysfunctional when pregnancy cannot be established, based on the presence of immune cells and their products, particularly cytokines. Some mis-conception is based on nomenclature and that the first discovery of biomolecules within the immune system and subsequently in the endometrium, resulted in assumptions of equivalent immune functions in the endometrium as in the blood. An example is that of uterine natural killer (NK) cells which have varying abundance in the endometrium, but are phenotypically and functionally distinct from the cytotoxic NK cells in the blood. As discussed earlier, this seems to be a point that is missed by many clinicians. Nevertheless, NK cell treatment has been widely used within fertility clinics at considerable cost to the recipients. Other immune therapies, including IV immunoglobulins, TNF α inhibitors, GCSF, PIF, intralipids and vitamin D supplements have been used to manipulate peripheral UK cells. There are no RCTs for such treatments and indeed, side effects including anaphylaxis, heart failure, induction of autoantibodies and lymphoma could be induced by such treatment [80–82]. We now await complete phenotyping of the immune cell populations within the endometrium of women with or without likely endometrial causes for their infertility. This will enable insight into the functional roles of these cells in infertility and whether or not these might be modified.

10.2 Endometrial Scratching

The ‘endometrial scratch’ has become very popular, since its first description in 2003 [83]. The original premise was that a newly repaired endometrium, would be more likely to attain receptivity: however, it is difficult to conceive that this could be the case given that menstruation and full regeneration of the endometrium occurs in every cycle and any site of injury and repair in the previous cycle would cease to exist after natural shedding. Multiple global studies examined a variety of patient groups with variable results: some supported the original findings but others did not. To date over 30 clinical trials have been reported, with reported outcomes ranging from implausible benefit to significant harm [84]. There was considerable heterogeneity among these studies, particularly regarding the selected population, type of treatment, and even timing and devices used to perform the endometrial injury. For example, the timing of the endometrial injury occurred over a wide time-span from day 3 of the preceding cycle, to day 3 in the cycle of transfer. Importantly, none of the studies reported improved reproductive outcomes in terms of live

birth rates following endometrial scratching. The majority of RCTs investigating endometrial scratching have methodological issues [85]. The most recent report on 1364 women undergoing IVF with additional subgroup analysis showed no benefit [86]. The procedure can be painful and was discontinued in a number of subjects for this reason. To the authors’ knowledge, no study focussing on older women has been reported.

10.3 Vasoactive Drugs

Conceptually, it has been thought that as vasodilators widen the lumen of blood vessels and increase blood flow, their use may cause uterine relaxation, increase endometrial blood flow and hence improve endometrial receptivity. A recent Cochrane review [87] examined 25 trials of vasodilators in IVF clinics. While they do appear to increase endometrial thickness (a logical conclusion) no effect on live birth is reported as most studies did not record this. Importantly, a number of adverse effects have been recorded, particularly with sildenafil (Viagra) [88], while other interventions with the same intent, (aspirin, heparin) also showed no significant benefit [79–82,89].

10.4 Platelet Rich Plasma

Reports of administration of autologous platelet rich plasma (PRP) in various clinical situations has increased over the past decade. For example, PRP stimulates cellular processes involved in endometrial regeneration [90] and has recently been administered via the uterine cavity, to treat women with refractory thin endometrium in IVF cycles using a range of protocols (for example, [91]). While it does appear to increase endometrial thickness, the literature remains very limited, the PRP preparations are not standardised and no large clinical trials have been performed [92]. Thus, the use of PRP remains experimental [93].

11. Conclusions

The endometrium is highly dynamic with its regular cyclical shedding and regeneration during a woman’s reproductive life, followed by quiescence during the menopausal years. The endometrium is essential to the establishment of pregnancy. If endometrial development is not synchronous with that of the pre-implantation blastocyst, implantation and subsequent placentation cannot take place. While the endometrium has been largely ignored in infertility clinics where the emphasis has been on developing a viable embryo for transfer, its importance is now recognised and the molecular and cellular changes essential to implantation are being studied in depth using new technologies. Regrettably, a plethora of new tests and adjuvant therapies are being offered to women, at substantial financial cost (see also [94]), yet few of these are of proven efficacy.

BOX 1. Take Home Messages

- The endometrium is essential for establishment of pregnancy and its successful completion.
- The endometrium is shed monthly at menstruation and then regenerated in preparation for a subsequent conception cycle.
- Following ovulation, the endometrium undergoes individually-timed differentiation of all its cell types and implantation can only occur if this is fully synchronised with early embryo development.
- Considerable and essential embryo-maternal molecular signalling occurs throughout the implantation process.
- Endometrial differentiation can be affected by many factors, hormonal, paracrine, immune, BMI and probably aging, along with signalling from the embryo.
- None of the adjuvants to reproductive technologies provided by clinics, are proven to be beneficial and should not be used.
- New technologies are enabling greater knowledge of the molecular processes underlying implantation and placentation.
- Currently there are no tests available that can accurately predict whether an embryo will implant in a particular cycle.

Author Contributions

The authors together planned content and order of the manuscript. LS took major responsibility for writing and managing the manuscript. ED contributed sections, reviewed and approved the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflicts of interest.

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