The human endometrium as a fertility-determining factor

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Intensive research work has been performed to better understand the regulation of the endometrium and its clinical implications to improve implantation. Although many proteins and molecules may influence endometrial development, their co-ordinated contribution to the implantation process is still poorly understood and a translation into clinical use has not sufficiently been performed. Clinical evaluation of the endometrium by ultrasound and other techniques, like endometrial biopsy and analysis of uterine secretions, has been intensively studied and therapeutic options to improve endometrial function have been suggested and tested. Systemic treatment with heparin, aspirin or corticosteroids did not result in improved implantation rates. Gene therapy and cervical treatment, e.g. with seminal plasma, are still in the phase of clinical research. Therefore, this review focuses on different aspects of endometrial research, which all contribute to the diagnosis, evaluation and therapy of endometrial function and dysfunction. First, the endometrial development towards a receptive milieu is described. Second, the actual clinical evaluation of endometrial receptivity, possible therapeutic strategies and in particular, the evaluation of endometrial function in the non-natural situation of hormonal stimulation is critically evaluated. In conclusion, the endometrium shall be considered as an important fertility-determining factor and therapeutic options should be developed in near future.

Key words: endometrium/implantation/infertility

Introduction

The implantation process of the human embryo requires a subtle dialogue between the mother and the embryo. On the maternal side, a so-called receptive endometrium is a prerequisite (Giudice, 1999). Although implantation itself is a dynamic process occurring between blastocyst and endometrial layers, the priming of the endometrium towards the window of implantation (WOI) is of pure maternal origin. A reduced endometrial receptivity is found in an increasing number of unexplained infertilities (Lessey et al., 1991; Dominguez et al., 1999). Therefore, an inadequate endometrium can be considered as a main fertility-determining factor.

The endometrium undergoes precisely defined morphological changes until a receptive endometrium is developed. These morphological changes were described as early as 1950 by Noyes, Hertig and Rock and occur under the control of the sexual steroid hormones, estrogen and progesterone. Estrogen is the dominant hormone of the proliferative phase (Hoozemans et al., 2004), whereas progesterone is the determining hormone of the secretory phase, where transformation and stromal decidualization occur.

Preparation of the endometrium is directed towards the cycle phase of receptivity, which is known as the WOI. The WOI is described as the period in the mid-luteal phase from day 19 to day 24, when implantation can take place (Navot et al., 1991; Dominguez et al., 2003) and is time limited (Wilcox et al., 1999).

Different cell types, which interact to allow a co-ordinated invasion, form the endometrial net. Four groups of resident endometrial cells can be differentiated: stromal cells, epithelial and endothelial cells, and non-resident immune cells. Stromal cells primed by estrogen will differentiate to decidual cells under the influence of progesterone. In vitro decidualization can also be initiated by the ‘downstream’ messenger cyclic adenosine monophosphate (cAMP) (Popovici et al., 2000; Gellersen and Brosens, 2003). Epithelial cells are involved in the adhesion process of the embryo. Receptivity of epithelial cells includes structural changes of the plasma membrane (Murphy, 2000) and the cytoskeleton (Thie et al., 1995; Martin et al., 2000), causing a transformation of microvilli at the implantation site simultaneous to the stromal decidualization process. Also, at the time of decidualization, increased cytokine production leads to the migration of immune cells into the endometrium, of which the most important are monocytes and uterine natural killer (NK) cells (King, 2000). Immune competent cells have their specific role in the preparation of endometrial receptivity. Besides cellular changes, decidualization creates an extracellular matrix (ECM) composition of utmost importance. This matrix is composed mainly of laminin, heparan sulphate proteoglycans and type IV collagens (Ferenczy and Giudice, 1995).

In the last two decades, a large number of proteins and other molecules, which are expressed in the endometrium in a cycle-dependent manner, have been described. Some of them, such as the insulin-like growth factor binding protein-1 (IGFBP-1), are used
as a marker for decidualization at least in in vitro experiments. Nevertheless, the importance of these factors for endometrial regulation, and the interaction of these factors, is far from being understood. Furthermore, new technologies of molecular genetics, in particular the microarrays (Popovic et al., 2000; Carson et al., 2002; Kao et al., 2002; Dominguez et al., 2003; Giudice, 2003; Riesewijk et al., 2003), have shown an enormous number of genes being expressed differentially within the endometrium throughout the cycle. However, the actual functions of these individual genes often remain to be determined.

Although knowledge on molecular mechanisms in the endometrium has increased tremendously, translation of this basic research into daily clinical routine is rather limited. Therapeutic options remain poor.

Therefore, the main purpose of this review is the re-evaluation of the endometrial contribution to fertility. The first section focuses on the endometrial development towards a receptive milieu. To elucidate the possible function of regulatory factors for each phase of endometrial development, we have used a cycle-dependent description. The second section is dedicated to the actual clinical evaluation of endometrial receptivity, possible therapeutic strategies and, in particular, the evaluation of endometrial function in the non-natural situation of hormonal stimulation.

The endometrial cycle—towards uterine receptivity

Every phase of the endometrial cycle is precisely tuned by many known and unknown genes that are regulated by endocrine, paracrine and autocrine factors. In this section, we have concentrated on some of the more important factors, many of which have been shown to be of relevance in humans. These factors are summarized in Figure 1. Microarray profiling of endometrial genes may allow a more comprehensive overview of gene encoding factors that are involved in endometrial differentiation. For example, osteopontin, apolipoprotein D, glycodelin A and Dickkopf/DKK1 are up-regulated, whereas olfactomedin-related estrogen receptor (ER)-localized proteins are down-regulated (Horcajadas et al., 2004). Furthermore, expression profiling of genes during the WOI in endometriosis may be helpful in identifying genes responsible for implantation failure (Kao et al., 2003).

The proliferative phase

The main feature of the endometrial tissue during the proliferative phase is active proliferation and angiogenesis to ensure nutrition of the developing new tissue, while suppressing apoptotic factors. Adequate development of endometrial tissue during this phase of the cycle is crucial for synchronization of the maturation process necessary for implantation during the secretory phase endometrium (Hoozemans et al., 2004). One of the key players during the proliferative phase is the rising female hormone estrogen. Estrogen is found to induce the proliferation of cells and demonstrates an indirect effect by stimulating the expression of steroid receptors such as the progesterone receptor (PR), which is crucial for progesterone action during the secretory phase, and ERs (ERa, ERb) (Chauhereau et al., 1992), as well as retinoic acid receptor (Deng et al., 2003) and androgen receptor (Slayden and Brenner, 2004).

Estrogen also acts as a cell survival factor in the late proliferative phase via inhibition of the tumour suppressor gene phosphatase and tensin homologue (PTEN). This gene is found to increase apoptosis and contact inhibition in endometrial cells by regulating various factors. PTEN inhibition on the other hand leads to elevated expression of such factors, e.g. Akt, a cell survival factor which acts via down-regulation of FasL, cell cycle inhibitor p27, cytochrome C release and many others (Uegaki et al., 2005). Anti-apoptotic genes like Bcl-2 are also up-regulated in the proliferative phase (Kayisli et al., 2004). These changes partially reflect the interaction between immune cells and the endometrial cells during this phase. Furthermore, ovarian hormones extensively regulate the growth factor system of insulin-like growth factor (IGF) and IGF-binding protein; induction of IGF-1 by estrogen during the proliferative phase (Giudice et al., 1993) is thought to contribute to the proliferation of the endometrium.

In a recent microarray analysis, additional genes within the human endometrium were discovered to be regulated by estrogen (Punyadeera et al., 2005). These include the tropho peptides, mammaglobins, wnt family and more. The majority of genes (65%) were down-regulated in late-proliferative-phase endometrium compared with menstrual tissue. They include the genes for many cytokines, enzymes involved in eicosanoid biosynthesis and immunomodulators and their receptors, all of which are active during the menstruation process via tissue degeneration, inflammation, hypoxia, epithelial repair and angiogenesis (Punyadeera et al., 2005).

The epidermal growth factor (EGF) system, including the EGF receptor (EGFR) together with its ligands EGF, transforming growth factor alpha (TGF)-α, platelet-derived growth factor (PDGF) and others, is highly regulated throughout the menstrual cycle; there is an elevated expression of human EGF receptor 1 (HER1), TGF-α and amphiregulin during the proliferative phase, where they act as mitogens for epithelial cells from the basal layer (Chau et al., 2004; Ejskjaer et al., 2005).

The macrophage migration inhibitory factor (MIF) is predominantly expressed in glands and surface epithelium in the late proliferative phase and premenstrual phase when architectural changes

![Figure 1. Up- and down-regulated factors of the proliferative and secretory phase.](image-url)
of the endometrium take place (Kats et al., in press). This is not surprising as MIF is a multifunctional cytokine that is involved in angiogenesis and tissue remodelling.

In a highly proliferating tissue like the endometrium during the proliferative phase, angiogenesis is mandatory to supply the tissue with sufficient nutrients. Angiogenesis takes place throughout the menstrual cycle with a significant increase in the basal layer and in the subepithelial plexus during the first part of the cycle. Angiogenesis changes in aspect during the cycle: major vessel elongation dominates the proliferative phase, whereas intussusception is the main mechanism during the early- to mid-secretory phase (Gambino et al., 2002). Not surprisingly, angiogenesis is regulated by many factors, such as nitric oxide, matrix metalloproteinases (MMPs) and many growth factors, including fibroblast growth factor, epidermal growth factor and vascular endothelial growth factor (VEGF), which is one of the key players in angiogenesis (Print et al., 2004).

The angiogenic factor VEGF seems to have additional functions in epithelial cells late in the proliferative phase, where it is found to have a proliferative effect (Hastings et al., 2003) even though the typical VEGF receptors are not found in this cell type. This effect could potentially be transmitted via an atypical VEGF receptor called neuropilin, which is simultaneously up-regulated within these cells (Germeyer et al., 2005).

In addition to their effect during menstruation, MMPs are thought to be relevant in tissue regeneration. MMP-7, for example, is up-regulated during the early proliferative phase (Hirota et al., 2003). Not surprisingly, angiogenesis is regulated by many factors, such as nitric oxide, matrix metalloproteinases (MMPs) and many growth factors, including fibroblast growth factor, epidermal growth factor and vascular endothelial growth factor (VEGF), which is one of the key players in angiogenesis (Print et al., 2004).

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Finally, several factors that are up-regulated during menstruation need to be eliminated in the proliferative phase. One example is the clearance of the high levels of prostaglandins (PGs) via increased expression of prostaglandin transporter (PGT, which acts to transport PGE2, PGD2 and partially thrombomaxan B, therefore contributing to the clearance and degradation of PGs (Kang et al., 2005).

To summarize, the proliferative phase is characterized by a concert of factors directed towards tissue remodelling, proliferative and anti-apoptotic processes, and stimulated angiogenesis.

The secretory phase, decidualization and implantation

In humans, during the late secretory phase of normal menstrual cycles, the morphological changes that characterize decidualization are taking place independently of conception. All cell types in the endometrium are affected, with the functionally distinct tissue showing characteristic endometrial cell differentiation and an infiltration by large numbers of immune cells. Progesterone, which suppresses proliferation and induces cell differentiation, is the major player during this second half of the menstrual cycle, and acts at least partially via the progesterone type A receptor (PR A, Wang et al., 1998; Bergeron, 2000). Furthermore, PR A acts as a repressor of PR B, which is the most transcriptionally active receptor. PR B is diminished in the glandular epithelial cells, but is still present in the stroma (Mote et al., 2000). Therefore, the action of progesterone during the implantation window is most likely through PR B in the stromal compartment. Recently, membrane progestin receptors have been described in the endometrium, indicating a possible action of progesterone in the endometrial glands via these membrane receptors (Fernandes et al., 2005). Epithelial cells are characterized by their glandular secretory transformation.

Stromal cell decidualization is accompanied by the production and secretion of distinct decidual proteins such as prolactin, IGFBP-1 and tissue factor (Irwin et al., 1989; Tseng et al., 1992; Christian et al., 2001). The endometrium in the mid- to late-secretory phase, as well as the decidua, is also infiltrated by a variety of bone marrow-derived immune cells. The predominant population (up to 70%) constitutes large granulated lymphocytes termed ‘decidual NK cells’ as well as T cells and macrophages. During the secretory phase, vascular remodelling occurs, with the main angiogenic mechanism being coiling and intussusception of the spiral arteries (Gambino et al., 2002). With these distinct morphological and biochemical changes, the endometrium is prepared for the implantation of the blastocyst. The embryo apposes and attaches to the endometrial epithelial cells and invades the endometrial stroma.

Apposition and adhesion

Pinopodes might influence the concentration of endometrial fluids near the implantation site, thus facilitating the process of adhesion and invasion (Stavreus-Evers et al., 2001). They can be found on the apical surface of the luminal endometrial epithelium and are strictly progesterone dependent. Scanning electron microscopy has revealed that pinopodes are found in 78% of endometrial biopsies on post-ovulatory day 6 in normally cycling women. The determination of the day on which fully developed pinopodes can be demonstrated in patients with repeated implantation failure in assisted reproductive cycles showed an increase in pregnancy rates when transfer cycles are modified accordingly (Nikas et al., 1999). Pinopode appearance, loss of steroid receptors and maximal expression of α,β3 integrin, osteopontin and leukaemia inhibitory factor have been shown in the same biopsy (Nikas and Aghajanova, 2002). This suggests pinopod formation as a possible, although because of the necessity of an invasive biopsy, impractical marker of receptivity (Bentin-Ley, 2000; Adams et al., 2002).

Furthermore, pinopodes show co-existence with the heparin-binding epidermal growth factor-like growth factor (HB-EGF), a member of the EGF family (Stavreus-Evers et al., 2002). Confocal microscopy experiments revealed the presence of HB-EGF both inside the luminal epithelial cells and on the surface of pinopodes, suggesting a possible role of HB-EGF in the attachment process. HB-EGF expression is increased in the mid-secretory phase, slightly preceding the expression of α,β3 integrins and HOXA10 (Lessey et al., 2002). There is a remarkable shift in HB-EGF expression during the menstrual cycle with maximal stromal expression in the proliferative phase and, in contrast, peak expression during the WOI in luminal and glandular epithelial cells (Leach et al., 1999).

The role of the EGF system in endometrium is not fully understood. This growth factor family acts via four specific receptors, HER1 to HER4. HER3 and HER4 are exclusively expressed in the secretory epithelium and glands (Ejskjaer et al., 2005). HB-EGF works through HER1 and HER4 and is considered as a potential implantation-promoting factor (Lessey et al., 2002). Data on EGF expression in the human endometrium throughout the cycle are contradictory. Whereas some groups were not able to demonstrate EGF in the endometrium regardless of the cycle phase (Ejskjaer et al., 2005), others describe EGF expression in a cycle-dependent manner in the epithelial cells (Imai et al., 1995) or in epithelium and stroma (Reis et al., 2005).

Mucins, in particular MUC1, are candidates for selection of the implantation site. A MUC1 barrier on the luminal epithelium
Impairs interactions between the embryo and the endometrium by anti-adhesive properties. Changes of MUC1 glycoforms and interactions with integrins may allow for a selected site of facilitated adhesion (DeLoia et al., 1998). Furthermore, the blastocyst itself cleaves MUC1 (very locally) and might influence the most appropriate site of implantation. In a clinical study, endometrial samples from women with idiopathic infertility have shown a reduced expression of the MUC1 D9B1 epitope compared with samples from normal fertile women (Aplin, 1991). This epitope was also reduced in women with the endometrium out of phase (LPD) as well as in women using an intrauterine device (IUD) (Aplin, 1991), suggesting that certain parts of the MUC1 glycoprotein are more important for implantation than others.

Integrins are the best-studied group of cell adhesion molecules in the endometrium. They combine heterodimeric, non-covalently bound α and β subunits. Their extracellular domain serves as a receptor for various ECM ligands such as fibronectin, collagen, and laminin (Albelda and Buck, 1990). The permanently expressed integrins on endometrial epithelial cells are α3β1, α5β1 and αvβ3. The only permanent one on stromal cells is αvβ3, the fibronectin receptor. Many integrins show a cycle-dependent expression. There is evidence that αvβ3 is the most important integrin during the implantation process (Lessey et al., 1992). It is co-localized with osteopontin and appears to be stimulated by EGF (Lessey, 2002). Several conditions associated with infertility seem to be accompanied by a low expression of integrin αvβ3, e.g. unexplained infertility (Lessey et al., 1995), luteal phase insufficiency (Lessey et al., 1996), endometriosis (Lessey et al., 1994a) and the presence of hydrosalpinges (Meyer et al., 1997).

Osteopontin is an important receptor for the integrins and indeed a possible function for embryonic implantation seems obvious. Osteopontin has been detected during the mid- to late-secretory phase (Apparao et al., 2001; von Wolff et al., 2001) in glandular epithelial cells and in uterine secretions from the secretory phase (von Wolff et al., 2001). It has an arginine–glycine–aspartic acid (RGD)-binding site that can bind to the transiently expressed α3β1 and α5β1 integrin heterodimers present during the implantation window (Lessey et al., 1994b). Consolidating its role in endometrial receptivity, microarrays analysing the WOI have shown that osteopontin is consistently up-regulated during the WOI when compared with both the late proliferative phase and the early secretory phase (Horcajadas et al., 2004).

The role of leukaemia-inhibitory factor (LIF) in implantation has been shown in a mouse model lacking a functional LIF gene; although the homozygous female mouse mated with a wild-type male the embryos did not attach and implant (Stewart, 1994). Data on humans, however, are still scarce. Clinically, more women with idiopathic infertility have undetectable levels of LIF in their uterine flushings in contrast with fertile women (Laird et al., 1997) and it has been shown that some infertile women have mutations in the coding region of the LIF gene (Giess et al., 1999). LIF and LIF receptor in human endometrium were first described by Cullinan et al. (1996). LIF mRNA expression is 3-fold higher in glandular epithelium compared with stroma and is cycle dependent with preferential expression in the secretory compared with the proliferative phase (Kojima et al., 1994).

The embryo expresses L-selectin after it hatches from the zona pellucida (Genbacev et al., 2003). L-selectin is expressed on vessel walls, where it captures leukocytes with their oligosaccharide structures and binds them after integrin activation at the site where they are needed (Alon and Feigelson, 2002). A similar process was postulated when Genbacev et al. discovered that highly O-glycosylated proteins, resembling the glycoproteins known to function as selectin ligands, were up-regulated at the beginning of the WOI. The hypothesis postulates that the embryo is transported through the uterine cavity by mucin flow. When it hatches, L-selectin binds oligosaccharides on the epithelial surface, slows down the blastocyst and enables a firm interaction with integrins, leading to embryo adhesion.

Furthermore, a large number of chemokines have been described at the embryo–maternal interface (Dimitriadis et al., 2005). For example the chemokine receptor CXCR1 is upregulated in epithelial cells in the presence of a blastocyst (Dominguez et al., 2003).

Invasion
Factors regulating the trophoblast. After traversing the epithelial cell layer, trophoblast cells of the implanting embryo invade the endometrial stroma with the goal of anchoring themselves tightly and invading the maternal vasculature in order to create a low-pressure circulation thereby accessing nutrients. Interactions of the invading trophoblast occur with the ECM and result in changes in trophoblast invasiveness. During the implantation process, ECM-degrading proteases (MMPs) are produced, mainly MMP-2 and MMP-9 (Bishoff et al., 1995; Kim et al., 1999). Both laminin and fibronectin, proteins of the ECM, are secreted by endometrial stromal cells predominantly in the secretory endometrium and decidua (Irwin et al., 1991). Laminin decreases prolactin and IGFBP-1 production in endometrial stromal cells in vitro (Brar et al., 1995), facilitating trophoblast invasion. Fibronectin has an RGD amino acid sequence, which enables it to bind to the fibronectin receptor on the differentiating invasive cytotrophoblast, probably also inhibiting its invasion (Damsky et al., 1994). Women with otherwise unexplained infertility or repeated abortions show different patterns of MMPs, tissue inhibitors of metalloproteinases (TIMPs) and cathepsin from normal controls (Jokima et al., 2002).

Another restraining endometrial factor which is secreted by decidualized endometrial stromal cells under the influence of progesterone is IGFBP-1 (Zhou and Bondy, 1992). IGFBP-1 is the best-characterized endometrial marker of decidualization besides prolactin. It has high affinity for the IGF peptides and acts primarily to inhibit IGF action on their target cells. At the implantation site, IGFBP-1 is believed to interact with the IGF-II produced by the cytotrophoblast (Zhou and Bondy, 1992). IGFBP-1 also has an RGD peptide sequence which binds αvβ3 integrin on the invading cytotrophoblast, thus inhibiting its invasiveness (Irwin and Giudice, 1998).

TGF-β is also cycle dependent, increasing during the secretory phase, with a maximal expression in early pregnancy decidua. It is equally produced in stromal and in epithelial cells (Chegini et al., 1994). TGF-β induces in the endometrium plasminogen activator inhibitor and TIMP-1 secretion, which inhibit trophoblastic plasminogen activators and MMPs, respectively (Graham et al., 1992). This suggests that TGF-β plays an inhibitory role on trophoblast invasion on the maternal–fetal interface.

Factors regulating immune cells. Colony stimulating factor-1 (CSF-1) is produced by endometrial epithelial glands as well as by macrophages during the mid-proliferative phase up to the mid-secretory phase (Tsoukatos et al., 1994). Its production declines
during the WOI (Pampfer et al., 1991). Its known function is in the development and growth of the decidual macrophages. It seems, however, to also have a differentiating effect on the first trimester trophoblast causing them to form a villous syncytial phenotype (Morrish et al., 1998). In patients, CSF-1 has been shown to vary largely in the serum of different individuals, making it difficult to compare with patient groups.

Glycodelin A is one of the most abundantly secreted glycoproteins in the secretory endometrium and early pregnancy decidua. It is produced by the endometrial glands and secreted into their lumen as well as into the circulation. Even though serum levels of glycodelin do not accurately reflect histological changes in the endometrium (McRae et al., 1991), new microarray data have shown that glycodelin A is one of the most consistently up-regulated proteins during the WOI (Giudice, 2004; Horcajadas et al., 2004). Its known function is to suppress the activity of NK cells (Okamoto et al., 1991). It also has an inhibitory effect on sperm binding to the oocyte’s zona pellucida (Oehninger et al., 1995).

Multiple studies have been performed measuring glycodelin in infertile patients either in serum or in uterine flushings. Uterine flushings seem to be more useful in assessing endometrial function than serum samples. However, standardizations and further investigations are needed to establish its future use in clinical practice (see Lindhard et al., 2002, for review).

Other factors of importance. Even though prolactin is one of the classic markers of decidualization and secreted abundantly by decidualized cells, its physiological role in the fetal-placental uterine compartment is not known (Tseng and Mazella, 1999).

Other factors which are regulated in the human endometrium during the cycle and are therefore believed to play a role in fertility regulation are the galectins. They have multiple functions, which are important in the process of implantation. They play a role in cell–cell adhesion, migration and chemotaxis, and they are important mediators of inflammation and have been characterized as major players in the defence of invading microorganisms (Almkvist et al., 2002, for review).

Galectins-1, -3 and -9 have been shown to be major players in the defence of invading microorganisms (Almkvist et al., 2002, for review). Galectins-3 and -9 are present in endometrial macrophages during the WOI (Popovici et al., 2005). Galectin-9 mRNA expression is ‘switched on’ during the WOI and increases further in early pregnancy deciduas (Popovici et al., 2005). Galectins-3 and -9 are present in endometrial glands, whereas galectin-1 is mainly expressed in the stroma (Popovici et al., 2005; von Wolff et al., 2005).

Glucose transporter proteins (GLUT) are essential for sustaining normal cellular function through oxidative metabolism. von Wolff et al. (2003) showed that GLUT1 increases in the secretory phase in the decidualized stromal cells significantly. Furthermore, GLUT1 mRNA is expressed at a significantly lower rate in endometrium from women with idiopathic infertility versus women with infertility because of tubal occlusion or male factor infertility. Even though present knowledge about the regulation of GLUT expression is poor, there is evidence that impaired GLUT expression in the reproductive tract is associated with defective fertility (von Wolff et al., 2003).

HOXA10 and 11 are well-known regulators of endometrial differentiation and are expressed throughout the menstrual cycle with a dramatic increase in the mid-luteal phase. Absence of HOXA10 has been shown to lead to implantation failure in knockout mice. However, the exact function is as yet unclear. HOXA10 seems to play a role not only in mice, but also in humans as endometrial HOXA10 expression is decreased in diseases associated with infertility, such as hydrosalpinx, endometriosis and the polycystic ovary syndrome (Taylor et al., 1999; Daftary and Taylor, 2002; Cermik et al., 2003). Patients with endometriosis do not show the mid-luteal increase of HOX gene expression. Women with hydrosalpinx, a condition known to alter implantation, show a reduced implantation rate in IVF. In vitro, HOXA10 expression is reduced if hydrosalpinx fluid is added to the culture medium (Daftary and Taylor, 2002).

Additionally, the cytokines interleukin (IL)-1, IL-11 and IL-15, as well as newly emerging chemoattractive cytokines, are important for the invasion process and have recently been discussed in an excellent review by Dimitriadi et al. (2005). They are involved in both the trophoblast regulation as well as in recruiting immune cells to the decidua during implantation.

Clinical assessment of endometrial function

Ideally, a technique to assess endometrial function and thereby predict endometrial receptivity must be easily performable within the daily clinical routine and would preferably be non-invasive. These requirements are met by ultrasound measurements of endometrial thickness and its echo pattern. More sophisticated techniques have been introduced as well to analyse certain aspects of endometrial function such as endometrial perfusion by endometrial Doppler studies, endometrial secretory function by biochemical assessment of serum and endometrial secretion and finally endometrial biopsies.

Ultrasound analysis of the endometrium

The two-dimensional ultrasound analysis of endometrial thickness was one of the first clinically relevant techniques to assess endometrial function. However, even though ultrasound of the endometrium is easy to perform and therefore the imaging modality of choice in a clinical setting, its prognostic value in determining pregnancy rate is low (Yuval et al., 1999; De Geyter et al., 2000; Kovacs et al., 2003; Zhang et al., 2005).

To improve the prognostic value of ultrasound analysis of the endometrium, Schild et al. (1999) and Raga et al. (1999) have performed an accurate endometrial volume calculation by three-dimensional ultrasound in patients undergoing IVF treatment. The results were similar to those by two-dimensional ultrasound, showing no relationship between mean endometrial volume and IVF outcome (Schild et al., 1999), but indicating a lower threshold under which implantation is reduced (Raga et al., 1999).

In patients treated with clomiphene citrate, data concerning pregnancy rate are controversial. Endometrial thickness is lower in cycles stimulated with clomiphene citrate than in natural cycles (Eden et al., 1989; Randall and Templeton, 1991; Nakamura et al., 1997), probably because of the interference of clomiphene citrate with ER kinematics in human endometrium (Birkenfeld et al., 1986). Dickey et al. (1993) found no pregnancies if endometrial thickness was <6 mm. However, the relevance of these data has been questioned by a study by Kolibianakis et al. (2004), indicating similar pregnancy rates in patients treated with clomiphene citrate with an endometrial thickness <6 and >6 mm. The irrelevance of endometrial thickness is also supported by Sterzik et al. (1997, 2000), who showed no correlation between endometrial histology and endometrial thickness either in spontaneous ovulatory cycles or in in vitro fertilization patients.

Endometrium and fertility
The ultrasonographic texture of the endometrium may have a greater prognostic value for implantation. In the proliferative phase, the endometrium has a hypoechogenic texture with a well-defined central line. This texture changes in the secretory phase, becoming hyperechogenic with no visualization of the central echogenic line (Takeuchi et al., 1991).

The triple-line multilayer appearance of the endometrium at the time of ovulation, because of luminal stromal density, has been described by several authors as a prognostic factor for pregnancy in gonadotropin-stimulated cycles (Bohrer et al., 1996; Oliveira et al., 1997). Other studies have focused on the sonographic appearance of the mid-luteal phase of the endometrium. Check et al. (2003) found significantly higher pregnancy rates in the group with a mid-luteal phase homogenous hyperechogenic pattern compared with a non-homogenous pattern. Similar data were described in patients with ovarian stimulation in IVF cycles (Check et al., 2000). Three studies evaluated the mid-luteal phase echo pattern compared with endometrial biopsy results (Grunfeld et al., 1991; Tani, 1992; Ficicioglu et al., 1995). Grunfeld et al. (1991) demonstrated a sensitivity of 100% and a specificity of 62% for the detection of histologically normal endometrial development. In those women where endometrial histology demonstrated asynchrony of the glands and stroma, the sonographic pattern correlated with stromal dating but not with glandular dating.

Studies with power Doppler energy (PDE) using the amplitude of the Doppler signal instead of its mean frequency shift to evaluate the perfusion of the endometrium are still controversial. Contart et al. (2000) and Baruffi et al. (2002) could not correlate analysis of endometrial vascularization by PDE with pregnancy rate. However, in a prospective study, PDE was a factor indicative of endometrial receptiveness (Yang et al., 1999). There was no association between endometrial thickness and PDE, supporting the above-mentioned suggestion that the thickness of the endometrium does not reflect its function. The different results of the studies might be because of a still unsolved problem: its quantification, which can only be solved by using specially designed computer software (Jimenez et al., 2005). Future studies will show whether the analysis of endometrial vascularization correlates to pregnancy rates. These future studies will also contribute to the discussion about the relevance of endometrial perfusion and angiogenesis in endometrial function, as endometrial angiogenesis seems to play an important role in endometrial function and implantation (Smith, 2001).

Analysis of serum and uterine secretion

Few studies have addressed the accuracy of the analysis of certain endometrial proteins in plasma or serum in predicting endometrial function. Joshi et al. (1986) for instance, suggested that the measurement of PP14, now glycodelin A, may be used as a non-invasive alternative to endometrial biopsy in the evaluation of adequacy in the luteal phase. However, a subsequent study by Mackenna et al. (1993) indicated that the measurement of serum concentrations of glycodelin A is unlikely to provide a reliable prognostic indication of endometrial function and of successful implantation.

The analysis of uterine secretions allows a direct evaluation of endometrial secretory function and is less invasive than endometrial biopsies. Three different techniques of evaluating endometrial secretory products have been introduced.

Licht et al. (1998) introduced an intrauterine microdialysis system, which not only allowed the analysis of endometrial cytokines and growth factors but was also used to study the effect of direct endometrial stimulation by applying HCG via the microdialysis device. However, as intrauterine microdialysis is very time consuming, it is not useful in the clinical routine setting.

Uterine flushings (Li et al., 1993), or aspiration of endometrial secretion (Beier-Hellwig et al., 1989; van der Gaast et al., 2003), are easier to handle and can provide valuable information about the function of the endometrium. However, contamination with small amounts of blood might influence the result of the analysis. Li et al. (1993) analysed the concentration of PP14 (glycodelin A) in uterine flushings in the mid-luteal phase and found a significant correlation between the histological assessment and PP14 (glycodelin A) concentration, suggesting this technique as a non-invasive tool for assessing endometrial function. Uterine flushings, performed in the luteal phase were also analysed by Mikolajczyk et al. (2003). In agreement with a reduced LIF expression in endometrial tissue samples of patients with multiple implantation failure (Hambartsoumian, 1998), uterine flushings from such patients also contained lower concentrations of LIF.

For clinical use, analysis during the phase of embryo transfer is of interest. Collection of secretion samples in patients undergoing IVF just prior to embryo transfer does not negatively affect pregnancy rates (van der Gaast et al., 2003), demonstrating the safety of this technique. Beier-Hellwig et al. (1989) and von Wolff et al. (1998) analysed uterine secretions collected during the early secretory phase to analyse the receptivity of the endometrium. However, as the prognostic value for the mid-luteal endometrial function and thereby for implantation is still limited, this technique has not been introduced in the routine clinical setting.

Analysis of endometrial biopsies

The analysis of endometrial biopsies is still the gold standard of endometrial evaluation. Histological dating of the endometrium (Noyes et al., 1950) and the highly sophisticated analysis of endometrial function, as described above, can provide detailed and physiologically relevant information. However, taking endometrial biopsies in the transfer cycle might be difficult and normally should be performed in a previous cycle. The reproducibility of endometrial analysis from one cycle to another is, therefore, of substantial interest to the clinician.

Endometrial biopsy and histological dating are by far the best examined endometrial parameters to determine luteal-phase defects. Several studies have addressed the interobserver variability of endometrial histological dating (Gibson et al., 1991; Smith et al., 1995; Myers et al., 2004) giving an interobserver variability of 20–40%. Because of these differences and the variability of endometrial development (Ishimaru et al., 1992), endometrial evaluation should be based on two consecutive biopsies in different menstrual cycles. Therefore, the prognostic value of endometrial biopsies might be limited. Consequently, Coutifaris et al. (2004) stated that histological dating of the endometrium during the routine evaluation of the infertile couple should be abandoned and the value of endometrial biopsies should be re-examined once molecular determinants are discovered and validated as markers of endometrial function.

In summary, clinicians can analyse endometrial function by ultrasound, endometrial secretion analysis or uterine flushing and
by analysis of endometrial biopsies. However, with the exception of the endometrial ultrasound analysis, which might give some prognostic and clinically useful information on the receptivity of the endometrium in hormonally treated individuals, all other techniques are still only of scientific interest. Many more studies are required to develop and validate clinically relevant prognostic markers for endometrial function.

Endometrial development under controlled hormonal stimulation

There is considerable evidence that high estradiol (E2) levels after ovarian stimulation impair the endometrial maturation and might negatively influence the implantation of the embryo. Clinical studies in an oocyte donation programme showed that E2 levels above 2500 pg/ml resulted in a significantly reduced implantation rate although embryo quality was not affected (Simon et al., 1995). Furthermore, pregnancy rates were significantly improved, if a step-down regimen of gonadotrophin stimulation resulting in lower E2 levels was compared with a standard stimulation protocol (Simon et al., 1998). However, in a large retrospective, comparative study between IVF/embryo transfer patients and recipients of donated oocytes, no negative impact of ovarian stimulation on embryo quality or pregnancy rate could be detected in patients with supraphysiological E2 levels (Levi et al., 2001).

A possible impact of hormonal stimulation on endometrial development is not restricted to the WOL. In a large number of carefully conducted studies, all of them based on histomorphological endometrial dating, a similar pattern of endometrial development throughout the menstrual cycle has been described. An excellent overview has been given by Bourgain and Devroey (2003).

Gonadotrophin and GnRH agonist/antagonist protocols

Most studies were performed in a standard GnRH analogue/gonadotrophin protocol.

In the late proliferative phase, premature secretory changes were found (Marchini et al., 1991). During the periovulatory period, endometrial maturation is generally advanced by up to 2–4 days (Ubaldi et al., 1997; Lass et al., 1998) with a reduced staining for estrogen and progesterone receptors (Bourgain et al., 2002).

Studies of the early post-ovulatory phase also observed an advanced pattern in HMG/HCG cycles (Garcia et al., 1984). However, later studies did not describe any difference between stimulated cycles and natural controls in this particular cycle phase (Barash et al., 1992; Macrow et al., 1994).

In the luteal phase, data on endometrial morphology in stimulated cycles are contradictory. Mid-luteal biopsies mostly reveal an advanced pattern combined with stromal–glandular disynchrony (Noci et al., 1997; Meyer et al., 1999; Basir et al., 2001). Seven days after hCG, endometrial glands show a reduced size and retarded emptying of secretory materials compared with controls (Basir et al., 2001). Small gland volume is associated with a reduced endometrial receptivity (Rogers et al., 1994). In contrast, Lukassen et al. (2004) could not demonstrate an increased endometrial advancement in IVF cycles 7 days after ovulation when compared with natural controls. If different groups of CD56 cells with positive or negative impact on implantation were compared, hormonal stimulation affected the CD56 cell ratio towards improved implantation conditions (Lukassen et al., 2004). Different findings might be at least partially explained by different effects of varying E2 levels. Moderate responders show diminished endometrial alterations compared with high responders (Basir et al., 2001).

A luteal support seems to be of importance for a regular endometrial development. Studies of IVF cycles with luteal support revealed normal in-phase histology irrespective of the type of luteal support (Bourgain et al., 1994; Ragni et al., 1999).

The late luteal phase shows normal endometrial morphology (Balasch et al., 1991). However, data are difficult to interpret because of different stimulation protocols and to substantial differences in luteal support, if any.

Gonadotrophin stimulation combined with GnRH antagonists also impairs endometrial maturation. At the day of ovulation induction, no premature advanced secretory transformation was detected (Papanikolaou et al., 2005). However, progesterone receptors were already significantly up-regulated when compared with natural cycles one day after the LH peak in glands and stroma, indicating an early onset of accentuated maturation. In 55 patients undergoing ICSI, an advanced endometrial development at the day of oocyte retrieval by an average of two and a half days was reported, and was correlated to LH levels before the administration of GnRH antagonists (Kolibianakis et al., 2002). In 10 oocyte donors, similar results were obtained (Kolibianakis et al., 2003). Interestingly, some patients with advanced endometrial dating of 2–3 days on the day of oocyte retrieval had a less pronounced advancement in the luteal phase, underlining the importance of early biopsy (Kolibianakis et al., 2003). When agonist and antagonist protocols were compared, no difference in advanced endometrial maturation at the day of oocyte retrieval and in the mid-luteal phase was visible (Saadat et al., 2004).

Clomiphene protocols

Negative effects of clomiphene stimulation on the endometrium have been described in IVF cycles and in ovulation induction cycles since the 1980s (Garcia et al., 1984; Sterzik et al., 1988; Rogers et al., 1991). In clomiphene citrate/HMG cycles, endometrial histology was still in the proliferative state a few days after oocyte collection (Abate et al., 1987). The endometrial anti-estrogenic properties of clomiphene citrate have been used as an explanation for low pregnancy rates after clomiphene citrate despite high ovulation rates. Classical clomiphene stimulation leads to a delayed histological dating (Palomino et al., 2005) and might lead to a reduction of pinopod formation in the mid-luteal phase (Creus et al., 2003). The glandular density is reduced and the number of vacuolated cells increases (Sereepapong et al., 2000).

A few studies have analysed the effect of estrogens to replace anti-estrogenic effects of clomiphene on the endometrial development. Vaginal estrogen gel from day 8 onwards and progesterone gel in the luteal phase fully prevent an out-of-phase endometrial maturation (Elkind-Hirsch et al., 2002). Ethinyl E2 exerts similar effects (Unfer et al., 2001). However, a possible impact on pregnancy rates has not been shown.

Molecular markers of endometrial development under hormonal stimulation

The IGFBP family in particular IGFBP-1 represents the best-characterized markers of the endometrium in the secretory phase.
Gene expression patterns under hormonal simulation

A genomic analysis of endometrial receptivity in natural cycles has been performed in several studies (Carson et al., 2002; Kao et al., 2002; Borthwick et al., 2003; Rieszewijk et al., 2003). However, the different situation of endometrial development under hormonal stimulation might yield a different gene expression pattern from normal cycles.

Horcajadas et al. have compared endometrial samples from fertile women undergoing ovarian stimulation for IVF at day HCG+7 with a previous natural cycle biopsy from day LH+7 in the same women. Ovarian stimulation produced significant differences to the natural cycle (Horcajadas et al., 2005). They identified 281 genes up-regulated in stimulated cycles more than 2-fold. In contrast, 277 genes were down-regulated. Genes of interest for the WOI in a natural cycle tend to be down-regulated after ovarian stimulation and vice versa. The results were confirmed by quantitative PCR experiments of seven differentially expressed genes (Table I). Moreover, other typical representatives of the WOI were detected amongst differentially expressed genes such as LIF (23.02-fold down-regulated) or IGFBP 1 (11.99-fold down-regulated). A comprehensive list is given by Horcajadas et al. (2005). However, the results are controversial. A similar study by Mirkin et al. found only minimal variations in gene expression after ovarian stimulation versus natural cycles (Mirkin et al., 2004). In particular, no difference for glycodelin A, LIF and integrins was found. Changes were different, when GnRH agonist and antagonist cycles were compared. Thirteen genes were up-regulated in the agonist group. Differences between both studies are difficult to interpret. Also progesterone supplementation does not seem to alter gene expression profiles in stimulated cycles (Mirkin et al., 2004). Different designs, selection of patients and stimulation protocols might explain differences. However, even though altered gene expression patterns in endometrium are found under different protocols, their implication is not yet known.

To summarize, the need for molecular markers besides endometrial dating is still a matter of debate in stimulated cycles, as markers like pinopod formation or integrin expression directly correlate to histological dating (Creus et al., 2003). Although there is evidence of severe changes in endometrial development in stimulated cycles their value for predicting clinical pregnancy rates is not yet known.

Therapeutic options in endometrial dysfunction and future trends

Maximum achievable pregnancy rates in IVF and ICSI cycles are ~50% per embryo transfer cycle (Gardner et al., 2000; Vlaisavljevic et al., 2001). Several studies have focused on technical factors such as embryo transfer conditions, which could be responsible for these limited pregnancy rates. It has been shown that ultrasound-guided transfer and treatment of vaginal infections can significantly improve pregnancy rates (Levi Setti et al., 2004). Other studies have analysed the relevance of autoimmune disease and maternal thrombophilia, such as factor V Leiden mutation, for successful implantation and found controversial results (Gopel et al., 2001; Martinelli et al., 2003). As an average pregnancy rate of 50% cannot be further increased by the improvement of embryo transfer and culture conditions or by optimal selection of blastocysts, the endometrial function and endometrial receptivity have been accepted to be major limiting factors in the establishment of pregnancy.

Previous studies revealed suppression of endometrial proteins such as LIF (Hambarstoumian, 1998), β3-integrin (Lessey et al., 1995), GLUT1 (von Wolff et al., 2003), osteopontin (von Wolff et al., 2001, 2004) and many others responsible for idiopathic

<table>
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<tr>
<th>Up-regulated</th>
<th>Down-regulated</th>
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<tr>
<td>Squalene epoxidase</td>
<td>Glycodelin A</td>
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<tr>
<td>Secretoglobin, family 1D, member 2 (plasma)</td>
<td>Glutathione peroxidase 3</td>
</tr>
<tr>
<td>Chemokine (C–X–C motif) legend 13</td>
<td>Transcobalamin I</td>
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<tr>
<td>Dipeptidylpeptidase 4</td>
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Table I. Differential gene expression after hormonal stimulation (Horcajadas et al., 2005)
infertility in patients. A new approach has therefore been suggested to improve implantation by direct treatment of dysregulated endometrial proteins. However, such treatments are based on a poor understanding of the complex endometrial interactions involving epithelial, stromal and endothelial cells, macrophages, lymphocytes and NK cells.

Three different treatment options are currently studied: first, systemic intravenous treatment, second, viral or liposome-mediated gene transfer by transcervical administration and third, stimulation of endometrium by transcervically applied protein cocktails. The first option, systemic treatment, was performed with heparin and aspirin to improve endometrial perfusion, prednisone to suppress fetal rejection, intravenous immunoglobulins to modulate endometrial immune cell function and recombinant LIF. However, even though small initial pilot studies were encouraging, large prospective randomized studies could not confirm the effect of these systemic treatment options on implantation rates (Stephenson and Flucker, 2000; Ubaldi et al., 2002; Stern et al., 2003).

The second treatment option, endometrial gene therapy, can be based on viral vectors or non-viral mechanisms. Even though the efficiency of non-viral mechanisms is less than that of viral vectors, it offers several advantages such as transient and therefore safer gene transfer, lower costs and easier handling (Daftary and Taylor, 2003).

In mice, liposome-mediated transfection has been successful in vivo. Mice uteri were transfected with the bacterial gene Lac-Z, a β-galactosidase (Charnock-Jones et al., 1997). Immunohistochemistry for β-galactosidase revealed expression in glandular epithelium and to a lesser extent by luminal epithelium. In another experiment, mice were transfected with HOXA10 antisense, resulting in fewer implantation sites (Bagot et al., 2000). Therefore, augmentation of HOXA10 expression by gene therapy has already been suggested as a future therapeutic option to improve implantation.

In humans, initial experiments have studied the applicability of transfection to human endometrial cells in vitro. Primary endometrial epithelial cells (Charnock-Jones et al., 1997) and Ishikawa cells (Bagot et al., 2000) were successfully transfected by using liposome-mediated gene transfer. First in vivo experiments were performed by transcervical administration of a lipoplex consisting of pcDNA3.1/Lac-Z on patients undergoing hysterectomy (Daftary and Taylor, 2001). These experiments and the first clinical trials using intracavitary gene therapy (Hortobagy et al., 2001) demonstrate the potential of gene therapy to treat endometrial dysfunctions. However, the success of such therapies will depend on the identification of still unknown single endometrial factors, which are essential for human implantation and which are dysregulated in patients with implantation failures.

The third therapeutic option, using transcervically applied protein cocktails, is based on the observation of a "post-mating inflammatory response" in mice (De et al., 1991; McMaster et al., 1992; Robertson et al., 1996). Seminal plasma, containing high concentrations of cytokines, growth factors and PGs (Gutsche et al., 2003), interacts with cervical and uterine epithelial cells and induces a surge in synthesis of cytokines, including granulocytemacrophage colony-stimulating factor (GM-CSF), IL-6 and an array of chemokines (Robertson et al., 1996). These pro-inflammatory factors stimulate the extravasation and infiltration of subepithelial stromal tissues by macrophages, dendritic cells and granulocytes.

In humans, intercourse elicits neutrophil recruitment into the superficial epithelium of the cervical tissues. In vitro studies revealed increased expression of endometrial epithelial proteins such as LIF and IL-6 by stimulation with seminal plasma (Gutsche et al., 2003). Seminal plasma apparently ascends through the cervix by uterine peristaltic contractions (Kunz and Leyendecker, 2002) or is transported from the cervix to the endometrium by unique vascular connections (Bulletti et al., 1997). These experiments and clinical studies, showing some increase in live birth rates in couples engaging in intercourse in IVF treatments (Tremellen et al., 2000), have led to the concept of using seminal plasma or its ingredients to stimulate endometrium in IVF cycles to improve implantation rate. This concept is currently studied by the authors in a randomized placebo-controlled double-blind study, applying either seminal plasma or placebo into the cervix at the time of follicle aspiration.

To summarize, many therapeutic options to treat endometrial dysfunction and thereby improve pregnancy rates have already been tested. Among those which promise to be efficient in the future are endometrial gene therapy and local endometrial stimulation. However, these therapies are still experimental and it will still take many years until they can be introduced into the clinical routine setting.

Conclusions

The endometrial development towards the WOI requires subtle collaboration of an extremely large number of different factors. Although many of them have been described, their individual function and their role in the network of endometrial development is not satisfactorily understood. Most studies lack functional experiments which are difficult to perform in the human system. Therefore, knowledge is still mostly restricted to descriptive experiments, in vitro data, or animal experiments. In the future, concentration on specific pathologies, like endometrial development in endometriosis or in women suffering from habitual abortions, might help to further clarify the role of the fertility-determining factors in the endometrium. Results from clinical studies such as that with seminal plasma, which are aimed at improving the endometrial prerequisites for implantation, may also prove to be useful. To our current knowledge, the endometrium, among many other things, is definitely a fertility-determining factor and the time has come to develop therapeutic concepts through clinical studies.

References


