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OPINION

Endometrial receptivity

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ABSTRACT

Embryo implantation depends on the quality of the ovum and endometrial receptivity. Endometrial receptivity is a temporally unique sequence of factors that make the endometrium receptive to embryonic implantation. Implantation window is a period during which the endometrium is optimally receptive to implanting blastocyst (D6-10 postovulation). No conclusive evidence of age related histological changes in the endometrium. The biochemical markers of endometrial receptivity include endometrial adhesion molecules (e.g. integrins), endometrial anti-adhesion molecules (e.g. mucin 1), endometrial cytokines, endometrial growth factors, endometrial immune markers and other endometrial markers. Integrins are the best markers of endometrial receptivity. Most interest has been focused on the av β 3 integrin since it appears in endometrial glands and luminal surface on D20-21. Endometrial function test may be the most efficient way to directly assess endometrial receptivity prior to undergoing expensive ART procedures as it can identify unreceptive endometrium. Pinopodes, are morphological markers of endometrial receptivity, which persist for 24 to 48 hours between days 19 and 21 of the cycle. Non invasive assessment of endometrial receptivity includes, high resolution transvaginal ultrasonography (US), three-dimensional US, Doppler US, three-dimensional power Doppler US, magnetic resonance imaging and endometrial tissue blood flow. Four strategies for improving endometrial receptivity: to develop ovarian stimulation protocols that cause a minimum reduction in endometrial receptivity or may even increase it; to avoid the endometrium during stimulated cycles, to improve uterine vascularization and to treat the pathology.
Key words: Endometrial receptivity, implantation, infertility

During the last two decades, several developments in controlled ovarian hyperstimulation (COH), fertilization, and embryo culture techniques have led to an optimization in the number and quality of embryos available for embryo transfer (ET) (1). In contrast, endometrial receptivity has failed to benefit from parallel improvements, and its disarrangement is likely to represent an important cause of the suboptimal embryo implantation rates observed in in-vitro fertilization (IVF)-ET.

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DEFINITION

Endometrial receptivity is defined as a temporary unique sequence of factors that make the endometrium receptive to the embryonic implantation (2). It is the window of time when the uterine environment is conductive to blastocyst acceptance and subsequent implantation (3). The process of implantation may be separated into a series of developmental phases starting with the blastocyst hatching and attachment to the endometrium and culminating in the formation of the placenta. The steps start with apposition, and progress through adhesion, penetration and invasion.

IMPLANTATION WINDOW

The endometrium is normally a non-receptive environment for an embryo, except during implantation window. Implantation window is a period during which the endometrium is optimally receptive to implanting blastocyst. Implantation of the human embryo may occur only during a regulated "implantation window" on days 6-10 postovulation, and surrounded by refractory endometrial status (2). It may be possible to artificially widen the implantation window by manipulating the pre- and peri implantation endocrine environment (4). Knowledge of the length of the human implantation window is of critical significance to all future studies aimed at identifying endometrial markers for endometrial receptivity (5). Unless we can identify when the uterus is receptive to the implanting embryo, it will never be possible to correlate changing endocrine, biochemical, and morphological endometrial parameters with receptivity. For optimal results in assisted reproductive technology (ART), it is critical to recognize the time for ET that would best corresponds with the implantation window. Embryo transfer data from assisted-conception cycles suggest a window lasting approximately 4 days, from days 20-24 of the cycle (1). The end-point of the window of implantation is more difficult to define in natural conception cycles. Data from assisted-reproduction embryo transfers indicate that the window for embryo transfer is about 4 to 5 days beyond which no progression can be achieved (5). Extrapolating this, with the knowledge that the window begins on day 20, we can surmise that the window for successful implantation ends at around day 24. This accords with data from in vivo studies of natural conception cycles where the first detection of HCG occurred between 6 and 11 days after ovulation (7,8). Further data from ovum donation cycles show that in late ET (day 19), successful pregnancy could still be achieved with first HCG detection possible on day 24. More recently we have turned to relating the window of implantation to the LH surge. The window occurs between LH+7 and LH+11.

ENDOMETRIAL STEROID RECEPTORS

It has been suggested that endometrial expression of estrogen receptors and progesterone receptors (PR) may be important in implantation. During luteal phase, progesterone causes loss of glandular epithelial PR, which coincides, with the time of implantation (9). This downregulation of PR is thought to be a critical step in a cascade of molecular events leading to implantation, with PR being abnormal in patients with luteal phase defects leading to infertility (10). A close correlation between PR downregulation and expression of pro-implantation integrins was described (11).

GENETIC FACTORS AFFECTING ENDOMETRIAL RECEPTIVITY

Many genetic factors are likely to be involved in the success or failure of implantation. The endometrial signature of genes during the window of implantation provides the opportunity to design diagnostic screening tests for patients with infertility and endometrial disorders and for targeted drug discovery for treating implantation-based infertility (12).

1. Female mice with Hoxa 10 removed exhibit uterine factor infertility, with normal ovulation and embryo formation but complete implantation failure (13). Hoxa 10 expression in the endometrium rises at the time of ovulation and has been shown to be essential for human implantation (14). The impact of Hoxagene expression in the endometrium of women receiving conception has yet to be evaluated.
2. Simmonds and Kennedy reported a novel gene, uterine sensitization-associated gene-1 (USAG-1), which is preferentially expressed in the maximal duration of endometrial receptivity (15).
3. Another new gene has been designated as endometrial bleeding associated factor (EBAF) found to be expressed in the late secretory and menstrual phase of the endometrium. Some insights are proposed for the role-played by this new gene in the endometrial preparation of implantation (16).

EFFECT OF HORMONES ON ENDOMETRIAL RECEPTIVITY

Estrogen and progesterone

Serum levels of estradiol (E2) appear to be of relatively little value in predicting endometrial maturation, although there is a correlation between endometrial thickness and serum estrogen levels in both natural and stimulated cycles (17). Estrogen levels alone express the activity of granulosa cells and not the maturity of the endometrium. The latter probably depends upon estrogen receptor development, which is genetically coded for each individual and, therefore, similar levels of estrogen can initiate different levels of endometrial maturity in different individuals. Levi et al (18), reported that exposure of the developing endometrium to supraphysiologic E2 level during COH does not inhibit endometrial receptivity, while Yang et al. (17), reported that elevated E2 may have a detrimental effect on endometrial receptivity.

Although it is known that excessive estrogen administered postovulation can prevent implantation (19), there is little understanding of how varying levels of estrogen and progesterone within the wide normal range, may influence receptivity. In study looking at 527 cycles in subfertile patients, it was found that significantly more viable pregnancies occurred among patients with an estrogen to progesterone ratio in the range of 7.36 to 12.22 (calculated as estrogen in pmol/L divided by progesterone in nmol/L). IVF has generated large amounts of accurate endocrine data on the circulating levels of estrogen and progesterone during stimulated cycles. In addition, receptive cycles are clearly identified by pregnancy following ET. As with the variability seen in the natural cycle, pregnancies have been achieved by numerous IVF groups using a wide range of stimulation protocols that have resulted in an even wider range of circulating estrogen and progesterone levels.

Gonadotropin Hormones

Bonnamy et al. (20), found that the uterine concentration of LH receptors and their occupancy by LH increased in the periimplantation period. This was explained by the authors as evidence for the role of LH in determining endometrial receptivity for implantation and subsequent decidualization. Edwards et al. (21), found that conception rates were higher in previously amenorrheic women than in cycling women, irrespective of age (48.4% versus 20.3%, respectively). This may be due to a beneficial effect of the higher gonadotropin levels on endometrial receptivity, in amenorrheic patients (22). This may be attributed to the presence of LH receptors in the endometrium (23).

GnRh agonist and GnRh antagonist

Low LH levels have been described after HMG treatment (24), after GnRh-agonist treatment (25) or after GnRh-antagonist treatment (26). These low luteal LH levels may lead to an insufficient corpus luteum function and consequently, to a shortened luteal phase or to the low luteal progesterone concentration frequently described after ovulation induction (27). A direct effect of the GnRh-agonist or GnRh-antagonist on human corpus luteum or on human endometrium, and thus on endometrial receptivity cannot be excluded, as GnRh receptors have been described in both compartments (28). Endometrial histology has revealed a wide range of abnormalities during the various ovarian stimulation protocols (29).

In GnRh-agonist cycles, mid-luteal biopsies has revealed increased glandulo-stromal dyssynchrony and delay in endometrial development, strong positivity of endometrial glands for progesterone receptors, decreased cell adhesion molecule profiles with early appearance of pinopodes. These changes suggest a shift forwards of implantation window. Progesterone supplementation improves endometrial histology, and its necessity has been established, at least in cycles, using GnRh agonists (30).

Endometrial Contraception

Modulation of endometrial receptivity is a promising approach for fertility since it allows a contraceptive to act specifically at the endometrium. Low dose antiprogestin administration has been proposed as a new modality to interfere with endometrial receptivity without disturbing ovarian function (31).

A number of antiprogestins e.g. mifepristone, onapristione, lilopristone, have been developed which compete with progesterone at the receptor level. In animal studies low dose antiprogestin (mifepristone) shown delayed development of endometrial maturation (32), The effect of mifepristone on the endometrium may be sufficient to prevent implantation. This effect is mainly due to interference with integrin distribution during implantation window (33). This may imply that the contraceptive effect of antiprogestin is primarily due to impaired endometrial receptivity. This approach of contraception still needs more studies.

Use as antiprogestin treatment after a single act of unprotected intercourse, and once-a-month treatment immediately after ovulation, have shown high contraceptive efficacy (34). Integrins is altered in glandular epithelium and stroma in women taking oral contraceptive pills. These alterations suggest that impaired endometrial receptivity is one mechanism where by oral contraceptive pills exerts their contraceptive action (35).

EFFECT OF AGE ON ENDOMETRIAL RECEPTIVITY

There is significant decline in human fecundity with advancing age. A significant decrement in success rate is also seen in older women undergoing assisted reproduction, including in-vitro fertilization (36). Rosenwaks et al., (37) have observed a drop in the ongoing pregnancy rate per ET, from 48.8% in women aged < 30 years to 13.6% in women aged < 42 years. Embryo implantation rates also decline in a linear fashion, from 29% in women < 34 years to approximately 5% at age 42. Borini et al., (38) found reduced pregnancy rates in patients over the age of 40. The abnormal endometrial receptivity in aging subjects may be due to decreased levels of progesterone receptors promoted by the low levels of E2 receptors. However, when the progesterone dosage for luteal support was increased, recipients aged over 40 years had a marked increase in pregnancy rate when compared with younger patients. Oocytes senescence is felt to be primarily responsible; however, some available data suggest that uterine factors, e.g. demised endometrial receptivity, may also play a role (39). Results of several clinical studies concerning ovum donation have shown that there is a decline in conception rate with increasing recipient age.

There are presently no treatment strategies apart from oocyte donation, which have been shown to significantly improve implantation efficiency in older women. However, recent efforts have focused on the continued development of improved stimulation protocols, facilitation of embryo implantation by zona pellucida micromanipulation (40), and the possibility of screening preimplantation embryos for aneuploidy (41).

There has been no conclusive evidence of age-related histological changes in the endometrium. Navot et al. (42), found no difference in either the pregnancy rate or the abortion rate between younger and older patients. Abdalla et al. (43), compared pairs of patients separated by at least 5 years of age receiving oocytes from the same donors. Their findings suggested no difference in implantation, pregnancy, miscarriage or live birth rates between younger and older patients.

POTENTIAL FUNCTIONAL MARKERS OF ENDOMETRIAL RECEPTIVITY

The period of maximal endometrial receptivity is marked by a wealth of coordinated morphological and biochemical events.

I. Biochemical Markers

Current theories of endometrial receptivity involve that through the luteal phase certain substances promote adhesion and certain substances inhibit adhesion (anti-adhesion). The presence of the former initiates the window of receptivity and the emergence of the latter closes this window (44). Both estrogen and progesterone regulate the activity of many growth and implantation factors. It is now clear that many of the actions of steroids in regulating endometrial function and preparation for implantation are mediated by locally acting growth factors and cytokines. These are secreted proteins that control cell functions such as proliferation, differentiation and secretion in a paracrine or autocrine manner.

Endometrial adhesion molecules

They include 4 main families: integrins, cadherins, selectins and immunoglobulin superfamily (45). There is little known about the role of cadherins or selectins in implantation. Integrins are cell adhesion molecules involved in cell-cell and cell-matrix interactions and contributing to cell migration and signal transduction (46). Integrins are a family of transmembrane glycoproteins that act as a receptor for extracellular matrix ligand, osteopontin (OPN). Three integrins are expressed by the endometrium with a pattern that coincides well with the window of implantation: $\alpha 1\beta 1$, $\alpha 4\beta 1$, and $\alpha_v\beta 3$ are coexpressed on glandular epithelium only during cycle days 20 to 24 corresponding the putative window of implantation. They have been proposed as the best of the immunohistochemical markers of endometrial receptivity during implantation window (47). Integrins are among the best-described markers of endometrial receptivity (46). Endometrial integrins are expressed in both epithelium and stroma. The reproducibility of integrin expression in the endometrium allows a complementary approach to histologic dating for the evaluation of endometrial receptivity. Most interest has been focused on the α_v β_3 integrin since this integrin appears in endometrial glands and luminal surface on cycle days 20 to 21, coincident with the opening of the window of implantation. Women with "out-phase" endometrium (luteal phase defect) fail to express the α_v β_3 integrin when biopsied during the window of implantation (48). Mid-luteal integrins was found to be lower in stimulated cycles than in natural ones, indicating a probable adverse effect from ovarian stimulation (49). This form of deficiency is termed as "type I defect. The vast majority of these patients, when treated with supplement progesterone have return to normal histologic endometrial maturation and normal α_v β_3 expression (50). Many infertile women with "in-phase" endometrium also fail to express the α_v β_3 integrin. These women have what must be considered occult endometrial receptivity defects, given that the pathologist finds these samples to be histologically normal. These have termed us a "type II defect", which has now been described among women with minimal or mild endometriosis (51), luteal-phase deficiency (52) hydrosalpinx and unexplained infertility. In women with endometriosis, it appears that α_v β_3 expression is reduced, while OPN expression is unaffected. Interestingly, binding of OPN to the surface epithelium appears quite limited when α_v β_3 expression is lacking (53). This indicates that endometrial dysfunction in some women may be the cause of reduction in cycle fecundity noted in these patients. As tissue sampling is inherently impossible in actual ET cycles, Reddy et al. (54) have reported that the expression of $\alpha 4$ and $\beta 3$ subunits on peripheral blood lymphocytes may correlate with endometrial cell integrin expression during the implantation window. This finding may be used as clinical marker to assess endometrial receptivity in infertile women. Moreover, frequent blood sampling advantageous over repeated endometrial biopsies, as the former approach is easier, non-traumatic and avoids intrauterine infections.

Endometrial anti-adhesion molecules

As the attaching embryo approaches the luminal epithelial surface of the uterus, it encounters a mucinous layer, the glycocalyx (44). The mucins in this layer are a group of anti-adhesive molecules, the most important of which is mucin 1(MUC-1). Mucins are a family of glycoprotein present on the surface of human epithelial cells. In human its expression is high during periimplantation period (55). It is possible that the high periimplantation levels of MUC1 could play a role in "shielding" the implanting blastocyst from other inhibitory factors on the epithelial surface. Alternatively, it could carry a specific recognition structure for the embryo. In women who suffer recurrent miscarriage there is evidence for reduced levels of MUC1 suggesting that these molecules play a significant role in the establishment and maintenance of early pregnancy (56).

Endometrial cytokines

It appears that all tissues involved in implantation (oocyte, embryo and endometrium) can synthesize cytokines (57). Although many cytokines may play a part in implantation, a vital

role has been clarified in four namely: Leukaemia inhibitory factor, interleukin-1, interleukin-11 and colony-stimulating factor (58). Leukaemia inhibitory factor (LIF) is produced by the receptive phase endometrium (59). Danielsson et al. (60) showed reduced immunostaining for LIF after treatment with the antiprogestin, mifepristone. These circumstantial evidences suggest that LIF plays a role in endometrial receptivity, but its exact role is currently unclear.

Endometrial growth factors

a. Heparin binding-epidermal growth factor (HB-EGF) was expressed during the time of maximal endometrial receptivity (61). Based on recent studies, it is tempting to think that HB-EGF maintains a role in both adhesion and development in the embryo (62).
b. Insulin like growth factor binding protein-1(IGFBP-1) is a major product of secretory endometrium and decediua. It inhibits the action of IGF at their target cells. Its role in endometrial receptivity awaits further investigation.

Endometrial immune markers

The endometrium has a large population of lympho-myeloid cells that undoubtedly play a variety of roles in the implantation process (63). It has been reported that women with unexplained infertility have significant lower levels of endometrial CD8+ (T suppressor/cytotoxic) and CD56+ (natural killer) cells, and higher levels of CD4+ (T helper/inducer) cells, than fertile control (64). The significance of these findings in relation to endometrial receptivity is unclear at present. One study examined the effects of endometrial large granular lymphocytes (LGL) cells on IVF outcome (65). The number of CD16 macrophage cells was significantly higher in the implantation group than the failed implantation group. The study only included small numbers and further studies are awaited.

Other endometrial markers

i. One of the many different substances that promote implantation is termed mouse ascites Golgi (MAG). The MAG test, done during an endometrial biopsy measures sticky mucinous substances secreted by endometrial glands before implantation and is considered as an endometrial function test (EFT)(66). Over 85% of the endometrial biopsies from normal, fertile women express higher levels of MAG between days 5 and 18 of the menstrual cycle with no expression after day 19. Approximately 70% with unexplained infertility showed abnormal MAG levels i.e. MAG was inappropriately expressed after day 19. Endometrial function test may be the most efficient way to directly assess endometrial receptivity prior to undergoing expensive ART procedures as it can identify unreceptive endometrium.
ii. The importance of extracellular matrix in endometrial function has been recognized. Laminin, fibronectin and collagen IV are found in secretory endometrium but are absent in the endometrium of patients with unexplained infertility (67). These suggest that these matrix proteins are likely to be required for implantation.
iii. Another endometrial protein, glycodelin, has a proposed immunomodulatory role during implantation. This protein is present in the endometrium under the control of progesterone and antiprogestins (68).
The usefulness of the molecular factors to assess endometrial receptivity remains to be proven. Studies performed to date have mostly included only small groups of patients with lack of fertile controls.
iv. Recently Dubowy et al, developed an EFT based on the endometrial expression of cyclin E and p27 (69). This test allows dating of the endometrium and differentiating between normally and abnormally developing endometrium. Cyclin E progressed from the basal to the lateral cytoplasm (midproliferative phase) to the nuclus (day 18 to 19) and was absent in biopsies after day 20. First appearing on days 17 to 19, p27 was found only in the nuclei.

II. Morphological Markers

Pinopodes

The endometrium undergoes a well-established series of histological and ultrastructural changes

under the influence of estrogen and progesterone during the menstrual cycle (70). Morphological changes include characteristic histological transformations, such as reduced mitotic activity, glandular secretion, and stromal edema, that are often accompanied by the presence of globular protrusions in the surface membrane of epithelial cells, named pinopodes (71). In addition, other modifications on the surface membrane of epithelial cells may occur, possibly including thinning of the glycocalix layer, as demonstrated in several animal species (72).

However, a number of different studies have thrown doubt on the functional importance of these morphological changes with respect to endometrial receptivity for implantation (73). Also, there is no conclusive evidence yet available to show that particular structural defect correlates significantly with reduced endometrial receptivity. Human studies using scanning electron microscopy (SEM) have proposed that these short irregular surface projections, or pinopodes, are transient markers of endometrial receptivity, which persist for 24 to 48 hours between days 19 and 21 of the cycle (74). Pinopodes appear on the apical surface of luminal epithelium around the 20th day of the menstrual cycle, and it has been suggested that pinopode formation might be a functional marker of uterine receptivity. Advanced endometrial histological features, in terms of dating (75) or in terms of earlier reduction in estrogen receptors and progesterone receptors, are correlated with the earlier appearance of pinopodes in stimulated cycles, further supporting the concept of a probable shift forward in the implantation window in these cycles (76). However, no experimental evidence is available to support this claim, while there is evidence of implantation occurring in the absence of pinopodes (77). With convincing evidence to the contrary, serious doubt remains on the obligatory requirement of pinopodes for successful implantation in the human.

Epithelial tight junction changes

For implantation to occur in the human, the embryo must breach the epithelium, raising the possibility that in the receptive uterus mechanisms may exist to reduce the integrity of the epithelial barrier. Freeze fracture studies have shown that epithelial tight junctions undergo a significant decrease in area between days 13 and 23 of the menstrual cycle (78).

Apoptosis

Apoptosis is a usual phenomenon throughout the menstrual cycle, peaking at menses, but locally regulated apoptosis is also vital for successful implantation. Recent evidence suggests that regulated apoptosis is important during the window of receptivity (79). On days 19-20, apoptosis is detectable in the glands of the basal layer, subsequently extending to the functional layer. The significance of this finding in relation to opening of the implantation window is under investigation.

NON INVASIVE ASSESSMENT OF ENDOMETRIAL RECEPTIVITY

Potential functional markers of endometrial receptivity, although promising, are expensive, invasive and circumstantial. It is therefore important to find alternative, non-invasive methods of assessing endometrial receptivity.

1. Transvaginal ultrasonography has been proposed as an alternative tool in the assessment of endometrial receptivity. It has been reported that endometrial thickness and pattern on the day before oocyte retrieval may be an indicator of the likelihood of achieving pregnancy (80). On other hand some authors found that endometrial parameters are not reliable predictors for pregnancy outcome in an IVF program (81).

    a. A good correlation between endometrial thickness and the prevalence of conception has been found (1,82). On the other hand other studies do not support this view (83,84). However, a very thin endometrium (<7mm) seems to be accepted as a reliable sign of suboptimal implantation potential (85). Implantation and pregnancy rates are significantly reduced if the endometrial thickness is increased (>14 mm) (86). This finding was not proved by other authors (87). Endometrial thickness has a significant positive

correlation with the duration of follicular stimulation, and an inverse correlation with age.
b. It was found that the multilayered echogenic pattern, the so-called triple line appearance, was predictive of pregnancy (83,84). However, pregnancies can occur in absence of this pattern, albeit at a lower frequency (83). Failure to establish a homogenous hyperechogenic pattern by the midluteal phase is associated with lower pregnancy rates (88).

2. With regard to uterine artery blood flow in stimulated cycles, equally controversial conclusions have been reached. Some workers have reported significant correlation between pregnancy rates and uterine artery Doppler flow values (89) while others have failed to show such a relationship (90,91) Schild et al., have reported no significant difference between conception and non-conception cycles with regard to uterine artery Doppler values (92). Uterine artery Doppler measurements are not representative of endometrial receptivity since they are based on flow to the entire uterus. Also spiral artery Doppler pulsatility index failed to predict implantation.

3. Raga et al. (93) performed three-dimensional volumetry of the endometrum at the time of ET to assess its value in predicting endometrial receptivity. The investigator found that a minimum volume of 2ml was a prerequisite for a receptive endometrium and that no pregnancy was achieved when endometrial volume measured <1ml. Beyond endometrial volume of 2ml, no relationship was apparent in terms of endometrial receptivity increasing if endometrial volume increased from 2-4 ml to > 4 ml.

4. In a recent study, Kupesic et al. (94) performed three-dimensional power Doppler ultrasonography of the endometrium on the day of embryo transfer, they concluded that endometrial thickness and volume, endometrial morphology and sub-endometrial perfusion can not predict endometrial receptivity. Use of subendometrial vascularization index was superior in predicting the pregnancy rate of IVF to using endometrial volume (95). Further studies are required to confirm these results.

5. For an embryo to implant, the quality of the endometrium as well as the (sub-) endometrial perfusion and vascularization may be more important factors than the global flow throughout the uterus (96), quantitative assessment of spiral artery blood flow and vessel density may allow further insight into endometrial receptivity. Recently, a novel way to assess endometrial receptivity for implantation has been conducted by using hysterofiberscopically laser blood-flowmetry to measure endometrial tissue blood flow (ETBF). It was concluded that ETBF is superior to conventional parameters for determining endometrial receptivity for implantation (97). Undoubtedly, the improvement of existing tools and the development of new noninvasive techniques are fundamental towards the adequate assessment and control of human endometrial receptivity.

6. Trunbull et al., have demonstrated the potential value of magnetic resonance imaging (MRI) in distinguishing conceptional and non-conceptional cycles (98). However, as a result of its high cost, MRI is unlikely to be incorporated into routine infertility practice.

PRACTICAL CONSIDERATIONS AND DILEMMAS

Practical considerations

The molecular concepts of implantation are fascinating (1). The use of marker proteins offer great promise for:

1. Better understanding of the process of both normal and abnormal implantation.
2. Providing clues to the causes and therapy of some types of early pregnancy losses.
3. Providing clues to the causes and therapy of some types of unexplained infertility.
4. Improving outcome and reducing the incidence of recurrent ART failure.
5. Providing new insights into contraception targeting the endometrium and embryo-endometrial interactions. Modulation of endometrial receptivity is a promising approach for contraception.

Practical dilemmas

Identification of one or more of endometrial parameters that definitely indicate receptivity for

implantation remains an elusive goal (99). Unfortunately, despite many well-documented endometrial changes around the time of implantation, it appears unlikely that obligatory markers for endometrial receptivity will be conclusively established in the near future. Furthermore, repeated tissue sampling is often required for their direct assessment. The relatively minimal clinical translation of the bulk of this basic scientific knowledge may be explained by several factors (1):

First, despite the physiologic importance of the events cited above, in humans, control of endometrial receptivity seems not to be as stringent as in some other species, and implantation can occur under a wide range of morphological and biochemical conditions (100). The implication of this is that no factor considered independently plays a determining role in the establishment of endometrial receptivity. Therefore, in an effort to assess the receptivity status of the endometrium, all of these factors should be investigated simultaneously, which is impractical for clinical purposes. Also, the embryo-uterus dialogue that takes part in the implantation process may further encumber the practical value of preimplantation endometrial measurements.

Second, tissue sampling, which is often required for the direct assessment of markers of endometrial receptivity, is inherently impossible in actual ET cycles. This biopsy may cause trauma and bleeding at the implantation site with a potential reduction in the chance of pregnancy. To avoid this problem, some centers advocate the performance of a mock replacement cycle, with timed endometrial biopsy in frozen or egg donation cycles (101). This, however, requires an additional preparatory cycle, which increases costs and is inconvenient to the patient. Given that the complex morphological, endocrine, and paracrine-autocrine interactions may undergo inter-cycle and inter-individual variations, it is difficult to extrapolate information obtained from experimental cycles.

On the other hand, Ubaldi et al (29) found that endometrial aspiration biopsy at the time of egg collection did not reduce pregnancy rates in women treated in IVF-ET. Recently, Olivennes et al, confirmed that uterine flushing on the day of egg retrieval during an IVF-ET cycle did not adversely affect pregnancy rates (102). These results should be confirmed in a larger sample of a prospective randomized study.

Finally, it is noteworthy that nearly all the morphologic and biochemical mechanisms that the uterus undergoes during its acquisition of receptivity are directly or indirectly regulated by ovarian hormones (103). Indeed, in IVF-ET with egg donation, sequential administration of physiologic doses of E2 and progesterone to women deprived of ovarian function has been shown to successfully restore endometrial receptivity and authorize the establishment of viable pregnancy (104). This indicates that the endometrium can be highly receptive as the exclusive result of physiologic hormonal replacement. Further, the outstanding pregnancy rates reported, which may be explained by optimum embryo and endometrial conditions that clearly surpass those commonly observed in conventional IVF-ET. A plausible explanation for the poorer outcome of conventional IVF-ET compared to egg donation is the possible adverse effect of COH used for conventional IVF-ET on the endometrium (105). In COH, administration of exogenous gonadotropins may exert, directly or through its supraphysiologic effects in ovarian hormones, unsuitable consequences on the endometrium, probably in proportion to the doses administered and the magnitude of the ovarian response.

STRATEGIES FOR IMPROVING ENDOMETRIAL RECEPTIVITY

Broadly speaking, there are four strategies that can be utilized for improving endometrial receptivity:

I. To develop ovarian stimulation protocols that cause a minimum reduction in endometrial receptivity or may even increase it.

i. There is statistical evidence that clomiphene citrate (CC) impairs endometrial receptivity and fetal development (106). Many IVF programs now recognize this fact and have moved to ovarian stimulation protocols that do not use CC. As of yet, however, consensus has not been reached that elimination of CC results in higher pregnancy rates, although reports have suggested it (107).

ii. Elkind-Hirsch et al. (108) investigated the possibility of correcting the endometrial alterations induced by CC by vaginal hormonal supplementation with estradiol (E2) and progesterone gel. They reported normalization of the alterations in endometrial morphology and improvement of endometrial receptivity in CC cycles and higher pregnancy rates.

iii. Exogenic 17 estradiol improving IVF outcome: A sufficient concentration of estrogen is necessary for endometrial proliferation during the follicular phase, for implantation and for progress of pregnancy (109). There is an increase in the efficiency of IVF if exogenic estrogen is used from the proliferative phase to early pregnancy. Exogenic estrogen during IVF cycles significantly increases both the implantation and the pregnancy rates and no difference in the thickness of the endometrium.

iv. Improving endometrial receptivity by decreasing estradiol levels, during the preimplantation period in high responders, with use of FSH step-down regimen. Controlled ovarian hyperstimulation is associated with supraphysiologic hormone levels compared with natural cycles (110). High E2 levels, which are known to be interceptive (18) and altered E2/progesterone ratios which are also associated with impairment of endometrial receptivity, are the main factors affecting receptivity in high responders (111). Estradiol levels on the day of HCG administration are significantly lower with the step-down regimen compared with the standard protocol. The implantation and pregnancy rates are better in step down regimen than in those resuming the standard protocol (112).

v. The early luteal phase of cycles undergoing controlled ovarian hyperstimulation is characterized by markedly elevated serum progesterone levels during the periovulatory period, advanced endometrial histological features, and an absence of endometrial pinopodes at the time of embryo implantation. Early progesterone rise has a negative impact on endometrial receptivity, but not on oocyte-embryo quality (113). These cause premature endometrial luteinization and premature appearance of implantation window, thus providing an explanation for the observed decrease in endometrial receptivity (75). Paulson et al. (114) reported that cycles with COH were associated with high early luteal progesterone levels and precocious secretory endometrium; and they suggested that low doses of antiprogesterone may correct the precocious luteinization and restore endometrial receptivity.

vi. Another approach that has been used to avoid the reduced endometrial receptivity that undoubtedly occurs following ovarian hyperstimulation is to return to natural cycle IVF, as was practiced when human IVF first started (115). While this strategy clearly avoids any reduction in endometrial receptivity associated with hyperstimulation, the disadvantages of working with a single developing follicle would seem to outweigh any advantages gained. There is clear evidence that IVF pregnancy success rates improve with the number of embryos transferred, at least up to a total of three. Thus, an optimal superovulation strategy should aim to produce at least three high-quality transferable embryos, to maximize the chances of success.

II. To avoid the endometrium during stimulated cycles altogether by freezing the embryos and replacing them in subsequent natural cycles (116). That it has not become a commonly used approach suggests that at present most IVF groups, either correctly or incorrectly, do not consider it advantageous. This may be for a number of reasons. The foremost of these is the belief that currently available freezing protocols cause a loss in embryo viability that will negate any beneficial effect that may build up from the increase in endometrial receptivity.

III. To improve uterine vascularization:

1. Low dose aspirin treatment significantly improves uterine and ovarian blood flow velocity, implantation and pregnancy rates in IVF patients (117). Low dose aspirin inhibits the synthesis of thromboxane A2 without affecting the synthesis of prostacyclin, thus explaining the increase blood flow velocity in uterine and ovarian arteries.

2. L-arginine (Nitric oxide donor): L-arginine supplementation improves the uterine blood flow, endometrial receptivity, implantation and pregnancy rates in comparison to a control group

(118). In addition, oral L-arginine improves endometrial thickness on the day of HCG administration.

3. Sildenafil (viagra): Nitric oxide relaxes vascular smooth muscle through cGMP mediated pathway and nitric oxide isoforms have been identified in the uterus (119). Sildenafil citrate is a newly developed, type 5-specific phosphodiasterase inhibitor that prevents the breakdown of cGMP and potentiates the effect of nitric oxide on vascular smooth muscle. Vaginal sildenafil may be effective for improving uterine blood flow and endometrial receptivity, implantation rate and pregnancy rate in IVF treatment with prior poor endometrial response. Nevertheless, larger studies remain necessary to confirm their effectiveness.

IV. To treat the pathological conditions:

1. Luteal phase defect: Because there is a suspected deficiency of progesterone in luteal phase defect, exogenous progesterone treatment has been utilized (120). A vaginal suppository containing 25-mg progesterone is inserted twice-daily starting 2-3 days after ovulation. Treatment is maintained until menstruation occurs or through the 10th week of pregnancy. Once pregnancy is diagnosed, a switch can be made to weekly injection of 17hydroxyprogesterone caproate (250 mg) through the 10th week. Vaginal administration accomplishes targeted delivery to the uterus without producing high circulating levels.

Dopamine agonist treatment has been reported to correct luteal phase defect associated with hyperprolactinaemia, but its value in women with normal prolactin levels has not been demonstrated (121).

2. Fibroids distorting the uterine cavity are associated with severe impairment of implantation and should be removed (122).

3. Intrauterine adhesions: Lysis under direct vision, by hysteroscopy is safe and more complete than blind curettage and improves implantation (123).

4. Uterine septum: Division of the septum by hysteroscopy is the treatment of choice (124).

5. Hydrosalpinx: The α_v β_3 endometrial integrin , which may mark the implantation window were expressed at significantly lower levels in women with hydrosalpinx and returned to normal after removal of hydrosalpinx (125). Disturbance of endometrial receptivity may be caused by altered chemical composition of the fluid from hydrosalpinx. The tubal fluid from a hydrosalpinx was lower in potassium and bicarbonate concentrations than normal tubal fluid (126). In addition, proteins specific to tubal fluid and total protein concentration in hydrosalpinx fluid were lower than in the fluid from non-diseased tubes. Also, hydrosalpinx caused inflammatory response that may be detrimental to endometrial receptivity or the developing embryo (127).

6. Endometriosis: In severe endometriosis, pregnancy and implantation rates are greatly reduced. Favorable results have been reported after prolonged pituitary-ovarian suppression using GnRH agonist for 3 to 6 months (128). This effect has been attributed to improvement of endometrial receptivity by the prolonged period of induced amenorrhea (129).

7. Autoimmune conditions: An increase in both implantation and pregnancy rates with predinosolone and low dose aspirin therapy in autoantibody positive women was demonstrated (130). A 10-mg daily dose of predinsolone is sufficient to improve the implantation rate. Because this dosage is too low to reduce autoantibody titers, the steroid effect may be derived from such mechanisms as an anti-inflammatory action or the regulation of immune cell (131). The role of natural killer (NK) cells in human implantation has recently attracted attention. Women with unexplained recurrent abortion and infertile women in whom multiple attempts at embryo transfer have failed, show elevated levels of peripheral and endometrial CD56+ CD16+NK-cells. Daily administration of predinsolone for 3 days has been reported to reduce the percentage of peripheral blood NK cells. It is possible that steroid had beneficial effect on IVF outcome through reduction of NK cells.

Recently, Barash et al demonstrated that local injury stimulates the endometrium in a manner that increases its receptivity for implantation (132). They reported that IVF treatment cycles preceded by endometrial biopsy doubles the chance for take-home baby. Further studies are required to confirm these results.

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