Ultrasound assessment of the peri-implantation uterus: a review

Y. ABDALLAH*, O. NAJI*, S. SASO*, A. PEXSTERS†, C. STALDER*, S. SUR‡,
N. RAINÉ-FENNING‡, D. TIMMERMAN†, J. J. BROSENS* and T. BOURNE*†

*Institute of Development and Reproductive Biology (IRDB), Imperial College London, UK; †Department of Obstetrics and Gynecology, University Hospitals, KU Leuven, Belgium; ‡Academic Division of Reproductive Medicine, School of Human Development, University of Nottingham, Nottingham, UK

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ABSTRACT

Emerging evidence suggests that early embryo implantation is a more active maternal process than hitherto appreciated, involving active encapsulation of the implanting blastocyst by maternal decidual cells and coordinated changes in the underlying inner myometrium, known as the junctional zone. These insights raise the possibility that early ultrasound markers predictive of adverse pregnancy outcome could be identified. In this review we assess the role of ultrasound in predicting the likelihood of different pregnancy outcomes and highlight potential novel markers that could be tested. Copyright © 2012 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Successful embryo implantation can take place only in a receptive uterus. In human beings, the uterus becomes receptive during the mid-secretory phase (days 19–23) of the menstrual cycle, a functional period widely referred to as the window of implantation (WOI)1–3. During this transient WOI, the endometrium becomes receptive during the mid-secretory phase (days 19–23) of the menstrual cycle, a functional period widely referred to as the window of implantation (WOI)1–3. During this transient WOI, the endometrium becomes receptive in order to support the implanting blastocyst. 

Decidual transformation of the stromal compartment occurs only in those mammalian species where implantation involves breaching of maternal tissues by the embryo8–9. Furthermore, a correlation exists between the extent of the decidual process and the depth of trophoblast invasion among various species7. Human placenta formation, however, involves not only trophoblast invasion of the maternal decidua but also of the inner layer of the myometrium, a highly specialized uterine structure known as the junctional zone (JZ). Importantly, trophoblast invasion of the decidua and JZ, which forms the placental bed, is not only interstitial but also intravascular9–11. In fact, this process of intravascular trophoblast invasion critically controls the transformation of highly-resistance, low-capacity spiral arteries into low-resistance, high-capacity vessels of pregnancy9,12. In other words, the decidua and JZ form a functional unit that largely determines the likelihood of successful pregnancy outcome.

The current model of fetal-maternal interactions views the trophoblast as the active invader of the passive decidua. However, this paradigm has been challenged by recent observations demonstrating that decidual cells acquire an invasive phenotype upon contact with the trophoblast13. Rather than being passively invaded, decidual cells may in fact actively encapsulate embryos that breach the luminal epithelium. Furthermore, emerging evidence suggests that decidual cells are programmed to respond to embryos of limited developmental potential, thus serving as biosensors that enable the mother to limit investment into failing pregnancies14. Furthermore, it has been suggested that impaired decidualization is associated with a prolonged WOI and lack of embryo quality control, thus facilitating implantation of developmentally compromised embryos14. This concept is supported by the landmark study of Wilcox and colleagues, demonstrating...
that implantation delayed beyond the conventional WOI is strongly associated with early pregnancy loss\textsuperscript{15}.

These observations suggest that prospective assessment of the quality of decidualization response in the endometrium may be an important tool for predicting the likelihood of successful implantation and pregnancy outcome. Since its introduction into the clinic, ultrasound has been used widely to assess uterine features such as endometrial thickness, endometrial pattern and uterine blood flow that may be predictive of pregnancy, especially in the context of assisted reproductive technology. Furthermore, the development of three-dimensional (3D) ultrasound and 3D Doppler studies now enables a much more detailed examination of uterine morphology, including visualization of the JZ\textsuperscript{16}.

In this review, we critically assess the role of various ultrasound techniques and markers in predicting the likelihood of conception and subsequent pregnancy outcome. In addition, we argue that, with the introduction of high-resolution ultrasound technologies, imaging of extremely early implantation events may yield novel markers predictive of pregnancy outcome.

Endometrial thickness

Several early studies assessed the value of endometrial thickness measurements by ultrasonography in predicting the likelihood of pregnancy. The data were conflicting. While the endometrium was reported to be thicker in conception cycles than in non-conception cycles\textsuperscript{17}, this was not confirmed by another study\textsuperscript{18}. In an in-vitro fertilization (IVF) population, endometrial thickness on the day after embryo transfer was reported to be higher in patients who subsequently conceived\textsuperscript{19}. In contrast, from a review of the early literature, it was concluded that endometrial thickness is comparable between successful and unsuccessful IVF treatment cycles (range, 8.6–11.8 and 8.6–11.9 mm, respectively)\textsuperscript{20}. The concept that the endometrium must measure at least 6 mm to sustain a pregnancy in natural cycles was first established two decades ago\textsuperscript{21}. This concept was subsequently refined by the recommendation that endometrial thickness should be ≥7 mm on the day of human chorionic gonadotropin (hCG) administration and ≥8 mm at the day 2 embryo transfer\textsuperscript{22}. These recommendations were supported by the observation that endometrial thickness of <6 mm has a high negative predictive value (NPV) for pregnancy\textsuperscript{20}. Thus, while ‘normal’ endometrial thickness does not necessarily predict pregnancy, a thin endometrium means that implantation is highly unlikely to occur.

Endometrial morphology

The appearance of the endometrium on ultrasound changes in a cycle-dependent manner. Consequently, endometrial morphology has been studied widely in an attempt to predict the likelihood of pregnancy. The results are similar to those of endometrial thickness in that a normal trilaminar appearance of the endometrium (multilayered or presence of midline echo) has a low positive predictive value (PPV) for pregnancy (33.1%), whereas the absence of a multilayered pattern does not exclude conception but renders it unlikely (NPV, 85.7%)\textsuperscript{20,23}.

Uterine blood flow

Uterine artery blood flow can be expressed usefully by impedance indices, the pulsatility index (PI) and resistance index (RI) (Table 1). Early studies in 1988\textsuperscript{24} reported that a poor uterine artery blood flow response to treatment with exogenous hormones (estradiol and norgestrel) was associated with a low pregnancy rate in IVF cycles. The authors hypothesized that a decreased uterine perfusion response is a contributing factor to infertility. In addition, they reported greater pregnancy rates after hormone therapy and improvement in uterine perfusion. This was in agreement with their previous study the same year, which showed increasing uterine perfusion with rising levels of plasma estradiol and progesterone\textsuperscript{22}. Taking this work a step further in 1992, Steer et al. reported higher pregnancy rates and implantation rates and more multiple pregnancies with lower uterine blood flow impedance before embryo transfer\textsuperscript{25}. They concluded that uterine artery blood flow is a useful method for assessing uterine receptivity in assisted conception programs\textsuperscript{27}. In agreement with these findings, other reports demonstrated that low impedance uterine artery blood flow in the early and mid luteal phase is associated with higher conception rates in assisted reproduction cycles\textsuperscript{23}, while high uterine artery impedance (PI > 3.3) predicts treatment failure with relatively high sensitivity but low specificity (88% and 26%, respectively)\textsuperscript{28}. In a review of the literature in 1996, Friedler et al. reported a high NPV and sensitivity (range, 88–100% and 96–100%, respectively) and a relatively higher PPV and specificity (range, 44–56% and 13–35%, respectively) with Doppler assessment of uterine artery blood flow using an upper limit for PI of 3 or 3.3, compared with the other ultrasonic parameters\textsuperscript{20}. These data suggest that uterine vascularity, or the factors that affect it, are important for implantation and for the subsequent pregnancy to be successful.

A recent study examined the optimal timing of B-mode and Doppler ultrasound evaluation of uterine receptivity in IVF populations. Different variables were studied, including endometrial thickness, endometrial morphology, uterine artery PI, the presence or absence of a protodiastolic notch, the presence of end-diastolic blood flow, and endometrial and subendometrial blood flow distribution patterns. The most effective combination for evaluating uterine receptivity was the presence or absence of end-diastolic blood flow in the uterine arteries, endometrial morphology and endometrial thickness. The best sensitivity and specificity were obtained on the day of hCG administration (81.1% and 81.3%, respectively)\textsuperscript{29}.


Sonography of the peri-implantation uterus

Table 1

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of patients</th>
<th>Population studied</th>
<th>Mode</th>
<th>Variables</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goswamy et al. (1988)</td>
<td>16</td>
<td>Spontaneous ovarian cycles</td>
<td>TA</td>
<td>Uterine artery PI, RI</td>
<td>Direct correlation with hormone assays</td>
</tr>
<tr>
<td>Goswamy et al. (1988)</td>
<td>153</td>
<td>Spontaneous ovarian cycles, then IVF</td>
<td>TA</td>
<td>Uterine artery flow velocity wave forms</td>
<td>Decreased uterine perfusion response may be a cause of infertility</td>
</tr>
<tr>
<td>Steer et al. (1992)</td>
<td>82</td>
<td>IVF</td>
<td>TV</td>
<td>Uterine artery PI</td>
<td>No implantation with PI &gt; 3 PL lower in successful then unsuccessful cycles</td>
</tr>
<tr>
<td>Steer et al. (1995)</td>
<td>86</td>
<td>GnRH cycle, then embryo transfer cycle</td>
<td>TV</td>
<td>Uterine artery PI</td>
<td>Higher conception rate with low impedance</td>
</tr>
<tr>
<td>Serafini et al. (1994)</td>
<td>96</td>
<td>Ovulation induction for IVF, GIFT and intrafallopian transfer</td>
<td>TV</td>
<td>Uterine artery PI, RI</td>
<td></td>
</tr>
<tr>
<td>Coulam et al. (1994)</td>
<td>405</td>
<td>IVF natural and donor cycles and IUI</td>
<td>TV</td>
<td>Uterine artery PI</td>
<td>Pl of &gt; 3.3 predicted 88% of non-conception cycles</td>
</tr>
<tr>
<td>Friedler et al. (1996)</td>
<td>495</td>
<td>Review of three studies</td>
<td>TV</td>
<td>Uterine artery PI</td>
<td>PI of 3 or 3.3: high NPV (88–100%) and sensitivity (96–100%) for conception</td>
</tr>
<tr>
<td>Habara et al. (2002)</td>
<td>121</td>
<td>RPL and controls</td>
<td>TV</td>
<td>Uterine artery PI</td>
<td>High impedance with RPL, especially with antinuclear antibodies</td>
</tr>
<tr>
<td>Raine-Fenning et al. (2004)</td>
<td>48</td>
<td>Normal cycles in subfertility and control</td>
<td>TV</td>
<td>Endometrial and subendometrial VI and VFI</td>
<td>Vascularity indices reduced in unexplained subfertility</td>
</tr>
<tr>
<td>Alcázar (2006)</td>
<td>725</td>
<td>Review of five IVF studies</td>
<td>TV</td>
<td>Endometrial and subendometrial VI and VFI</td>
<td>Conflicting results</td>
</tr>
<tr>
<td>Ng et al. (2007)</td>
<td>161</td>
<td>IVF</td>
<td>TV</td>
<td>Endometrial and subendometrial VI and VFI</td>
<td>VI and VFI higher with live births than in miscarriage</td>
</tr>
<tr>
<td>Lazzarin et al. (2007)</td>
<td>230</td>
<td>RPL and control</td>
<td>TV</td>
<td>Uterine artery PI</td>
<td>Higher PI in unexplained RPL, uterine abnormalities and antiphospholipid antibodies syndrome</td>
</tr>
<tr>
<td>Dechaud et al. (2008)</td>
<td>124</td>
<td>IVF or ICSI</td>
<td>TV</td>
<td>Uterine artery PI, paradiastolic notch, end-diastolic flow, endometrial and sub-endometrial flow pattern</td>
<td>End-diastolic blood flow with endometrial morphology and endometrial thickness gave sensitivity and specificity of 81%, PPV of 65% and NPV of 89%</td>
</tr>
<tr>
<td>Chen et al. (2010)</td>
<td>134</td>
<td>RPL and control</td>
<td>TV</td>
<td>Endometrial and subendometrial VI, FI and VFI</td>
<td>Low VI, FI and VFI in unexplained RPL</td>
</tr>
</tbody>
</table>

3D endometrial vascularity

3D ultrasound has been shown to have a low intra- and interobserver variability in assessing endometrial volume, with Virtual Organ Computer-aided Analysis being the most reproducible technique. Endometrial vascularity can be assessed also using 3D power Doppler. Using B mode and color Doppler imaging, the cyclical changes in uterine size, echogenicity and vascularity have been studied throughout the menstrual cycle in relation to a positive urinary luteinizing hormone test and day 1 of next menses. Endometrial thickness was found to increase up to days 3 and 4 of the cycle, after which it remains relatively constant. This was associated with a gradual decrease in the uterine arterial PI throughout the cycle, which significantly increased at the time of next menses. These cyclical changes have been further studied using 3D ultrasonography. Endometrial volume increases in the follicular phase and plateaus in the luteal phase. Vascularity of the endometrial and subendometrial regions increases from the mid-follicular phase and peaks 3 days prior to ovulation before decreasing again over the next 5 days and then increasing until the next cycle. Raine-Fenning et al. further showed that endometrial and subendometrial vascularity indices were significantly lower in women with unexplained subfertility during the mid and late follicular phase, irrespective of estradiol and progesterone levels. The subendometrial region was defined as a 5 mm shell around the defined endometrial contour. As would be expected from endometrial thickness data, 3D measurements of endometrial volume have showed a high NPV for implantation failure. However, there have been conflicting results among investigators regarding
differences in endometrial volume, as well as endometrial and subendometrial vascularity between conception and non-conception cycles\textsuperscript{30,37}. Several factors have been cited as possible explanations for these conflicting results, e.g., different ultrasound examination and analysis techniques, variations in the resolution of equipment and different treatment protocols, especially in the fertility groups\textsuperscript{39}. However, endometrial and subendometrial 3D power Doppler indices have been shown to have acceptable reproducibility in evaluating physiological and pathological changes of the endometrium\textsuperscript{38}. While Doppler studies of the uterine arteries have suggested a mechanism whereby uterine vascularity impacts on implantation, 3D Doppler studies of the endometrium have not supported this concept. Currently we do not know whether compromising endometrial vascularity leads to implantation failure.

A few studies have tested the hypothesis that changes in endometrial vascularity play an essential role in recurrent miscarriage (Table 1). Higher uterine artery PI was reported in women with recurrent pregnancy loss (RPL), especially in those with antinuclear antibodies, than in controls\textsuperscript{39}. Findings were similar in women with unexplained RPL or uterine congenital abnormalities and in women with antiphospholipid antibodies syndrome\textsuperscript{40}. Most recently the endometrial and subendometrial vascularity in cases of unexplained RPL were reported to be lower in women with RPL than in controls. This assessment took place 7 days post ovulation\textsuperscript{41}. It is of interest to observe the same patterns of endometrial vascularity in patients with RPL and failed assisted reproduction, suggesting that endometrial vascularity does play a role in endometrial receptivity and pregnancy maintenance.

### The uterine junctional zone

The JZ, or endometrial-myometrial junction (EMJ), is the transitional zone, sandwiched between the endometrium and the outer myometrium. Unlike most human tissues with a mucosa, the endometrium does not contain a submucosal layer. This layer usually exists to protect against mucosal invasion into adjacent tissue\textsuperscript{42}. Brosens et al. postulated in 1995 that the JZ differs from the outer myometrium not only structurally but also functionally. Furthermore, they proposed that irregular thickening of the JZ is the magnetic resonance (MR) criterion for diagnosis of diffuse adenomyosis\textsuperscript{16}. An increased diameter of the posterior JZ of the uterus on MR imaging correlated with invasion of the basal endometrium into the inner myometrium from as early as the third decade of life\textsuperscript{43}. The JZ is thought to play an important role in regulating uterine function and hence fertility\textsuperscript{44}. Video-vaginosonography studies have shown that propagated myometrial contractions in the non-pregnant uterus originate only from the JZ and that the frequency and orientation of these contraction waves are dependent on the phase of the menstrual cycle\textsuperscript{45}. These inner myometrial contractions vary in orientation, amplitude and frequency throughout the menstrual cycle, and are thought to be influenced by estradiol and progesterone. In the follicular phase of the cycle, these contractions are from the cervix towards the fundus and their amplitude and frequency increase significantly as ovulation approaches. Following ovulation a decrease in contractility under the influence of progesterone is observed\textsuperscript{46}. There is evidence that this pattern of contractions facilitates sperm transport\textsuperscript{46}, aids implantation of the developing blastocyst, improves the supply of oxygen and nutrients to the decidua\textsuperscript{47} and, in addition, contributes to menstrual shedding\textsuperscript{48,49}. On the other hand, alteration of the JZ interface, and hence contractility, is proposed to have an integral role in diverse reproductive disorders\textsuperscript{48}. Indeed, high-frequency uterine contractility in women undergoing IVF treatment on the day of embryo transfer has been shown to negatively affect the outcome, possibly by expelling embryos from the uterine cavity\textsuperscript{50}. It has been suggested that aberrant uterine peristaltic activity at the EMJ may cause local microtraumas that enable invasion of endometrial glands and stroma\textsuperscript{51,52}. While this proposed model of adenomyosis remains speculative, electron microscopy studies demonstrated that smooth muscle cells from uteri with adenomyosis are ultrastructurally different from myocytes of disease-free uteri\textsuperscript{53,54}. High-resolution MRI has been used to monitor changes in endometrial and JZ morphometry during the normal menstrual cycle. This analysis demonstrated that both endometrial and JZ volumes increase significantly towards ovulation. While the endometrial volume decreases significantly post ovulation, the JZ appears to be less regular during the luteal phase of the cycle\textsuperscript{55}. These findings support the hypothesis that the JZ and endometrium constitute a functional unit\textsuperscript{16}.

Changes in the JZ during pregnancy were first observed incidentally on MR scans carried out 7 days post ovulation in what turned out to be a conception cycle. Even this early in the pregnancy, a low-signal intensity mass at the site of implantation has been visualized, with changes in the regularity of the adjacent JZ\textsuperscript{56}. These uterine changes are not observed in cases of ectopic pregnancy\textsuperscript{37}. It was therefore postulated that these MRI features are the result of a change in local blood flow in the area underlying the implantation site, in response to the presence of implantation factors\textsuperscript{58,59}. The impact of the JZ structure on the likelihood of pregnancy after IVF treatment was recently evaluated in a prospective study. The uterus was imaged prior to IVF treatment and the average and maximal JZ thickness values were measured on T2-weighted sequences. Strikingly, the implantation failure rate was as high as 95.8% in women with an average or maximal JZ of 7 and 10 mm, respectively, compared to 37.5% in other patients ($P < 0.0001$). This strong association between an abnormally thickened JZ and IVF failure was independent of the cause of infertility or the age of the patient\textsuperscript{60}.

3D transvaginal ultrasonography now enables reliable assessment and visualization of the JZ. As on T2-weighted MR images (Figure 1), the JZ on ultrasound appears
Sonography of the peri-implantation uterus

Figure 1 The uterine junctional zone appearing as a low-signal area between the endometrium and the myometrium on a pelvic magnetic resonance imaging scan.

Figure 2 Three-dimensional rendered view of the uterus in the coronal plane showing the junctional zone as a hypoechoic area surrounding the endometrium.

Figure 3 Another three-dimensional view of the uterus in the coronal plane showing a regular junctional zone.

Ultrasound assessment of peri-implantation events

Although there is considerable interest in visualising the JZ for the diagnosis of adenomyosis, no studies have been reported to date on potential changes in JZ structure or morphology associated with early implantation events. Based on the observations outlined above, it appears reasonable to speculate that the JZ is exquisitely sensitive to pregnancy-associated hormonal and embryonic signals. If so, failure of the JZ to remodel in early pregnancy may be predictive of subsequent failure or even obstetrical complications such as preterm labor.

Unlike MRI, ultrasonography is not contraindicated in early pregnancy. As outlined above, 3D ultrasonography appears to be more accurate than conventional 2D imaging in characterizing changes in the JZ. Increased ultrasound resolution also means that it has become possible to visualize the probable site of implantation. Early implantation sites are usually characterized by the presence of a hyperechoic ring around the conceptus that protrudes into the endometrial lumen (Figure 4). This appearance on ultrasound fits well with the emerging concept that the implanting embryo is rapidly and actively encapsulated by migratory decidual cells. It remains to be seen whether the absence of decidual protrusion or encapsulation detected on ultrasound could serve as an indirect marker of inadequate decidualization, and thus predict subsequent pregnancy loss.
SUMMARY

There is a growing body of evidence supporting the notion that defects in the implantation process create an adverse ripple effect during the subsequent course of pregnancy, ultimately culminating in either pregnancy loss or obstetrical disorders associated with impaired placentation such as pre-eclampsia, placental abruption and fetal growth restriction. As outlined in this review, high-resolution ultrasonography has the potential to provide new insights into the implantation process and to functionally assess the quality of the maternal decidua response and associated, but as yet ill-defined, changes in the JZ. If this notion is correct, it should in turn be possible to predict pregnancy complications at the implantation stage and to design tailored interventions that would genuinely prevent these subsequent complications.

Areas for further research:

- Quantification of JZ by 3D ultrasonography: subjective assessment and pattern recognition, quantitative assessment and reproducibility
- Intermethod comparison of 3D ultrasonography and MRI for measurement of the JZ
- Accuracy of 3D ultrasonography and MRI in delineating the JZ and identifying abnormalities (histological test accuracy studies)
- Use of 3D ultrasonography to study early changes in the endometrium and the JZ associated with implantation
- Use of 3D ultrasonography to study the relationship between JZ features and infertility

REFERENCES


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