Endometrial Receptivity and Scoring for Prediction of Implantation and Newer Markers

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ABSTRACT

There are many markers to indicate the preparation of endometrium for successful implantation, and these manifest during the implantation window. Today TVS color Doppler can reliably show the increase in blood flows during the peri- and postovulatory phase and objectively assess these flows to predict endometrial receptivity.

Keywords: Ultrasound, Implantation, Endometrial receptivity, Uterine biophysical score, biochemical markers.

INTRODUCTION

Assisted reproductive technologies are here to stay. At 11.30 am, 25th July, 1978 with the birth of Louise Brown, infertility was conquered by in vitro fertilization and embryo transfer. In the last 20 years, we have over 30,000 IVF babies from over 500 IVF centers. In India, 20 IVF centers here contributed to around 5,000 pregnancies by this technology. With improvement in stimulation protocols, there is better understanding of the physiology and pharmacology of gonadotrophins, and with the advent of recombinant FSH, better monitoring facilities, state-of-art laboratory equipment and facilities for ICSI, TESA and MESA are now available at all centers. ART has come to stay as an important armamentarium of infertility management. Better ultrasound imaging, color flow measurements of blood flows in the uterus and vascularization of endometrium are now available. This has led to improved understanding of physiology of ovaries, uterus and endometrium during various phases of normal menstrual cycles, ovulation induction, super ovulation and controlled ovarian hyperstimulation, and implantation and normal pregnancy.

Based on numerous studies all over the world on color flow dynamics of reproductive organs, various scoring systems and criteria have been devised to predict implantation of the embryos transferred in ART. It is now only the implantation, which eludes scientific basis. To overcome this, various centers are increasing the number of embryos transferred, which often leads to multiple gestations and painful embryo reductions, and spontaneous early pregnancy loss. It becomes an absolute necessity to evaluate the uterus and endometrium by color Doppler imaging, so we can do embryo transfers in only favorable uteri. The fertilized embryos not used can be frozen for the use in a favorable cycle based on the various scoring criteria like UBP and USSR.1,2

PHYSIOLOGY OF IMPLANTATION

Implantation presents the greatest challenge to the ART specialist, in whichever form it is applied for. It is a known fact that less than one-third of human embryos replaced in the uterus complete implantation. The uterus also prepares for implantation along with the hatching blastocyst. The endometrium has to undergo a lot of changes, which will help in the process of implantation and will support the embryo. The endometrium undergoes the following changes:

1. Attachment of blastocyst to the endometrium
2. Penetration of blastocyst into the stroma.

The stroma will help by:

a. Anchoring
b. Sustaining the embryo
c. Control the invasiveness of trophoblast
d. Production of growth factors and substrates
e. Stroma responds to the proteins and hormones of ovary and embryo.

This physiological process of implantation is a well-organized and orchestrated process.

Implantation is an important step in establishing pregnancy and still scientific basis of failure of implantation eludes us. Implantation is difficult to study because our knowledge of what happens during first week of human life in vivo is very limited and to study this process is difficult, for it requires a blastocyst to interact with a receptive endometrium.3-5

A successful human implantation is an interaction of two separate processes: (1) Embryo development (2) endometrial differentiation. A synchrony between these functions is important and this produces a transient period of implantation known as nidation/implantation window.6
Extensive studies have been aimed to develop a specific marker for uterine receptivity and are based on:
1. Biochemical evaluation
2. Ultrastructure study of pinopodes
3. Ultrasound scoring systems

**BIOCHEMICAL EVALUATION AND MARKERS**

1. Cytokines
   a. Leukemia inhibitory factor
   b. Colony stimulatory factor
   c. Interleukin-1
2. Integrins
   a. Glycodelin
   b. MUC1

Many biochemical factors, important for human implantation, have been discovered and are being discovered. The following seven factors seem to be the most important biochemical markers for endometrial receptivity.

**Cytokines**

Leukemia inhibitory factor, interleukin-1, and colony stimulatory factor-1 are actively produced by the endometrium cells and they are shown to be important in the crosstalk between embryo and endometrium.

**LIF**: It is likely to influence preimplantation, implantation, embryo development and placentation. LIF can be screened in endometrial flushings and should prove to be a screening marker for endometrial receptivity.

**CSF-1**: Low levels of CSF-1 have been associated with recurrent spontaneous abortions. Increased production of CSF-1 is expressed throughout preimplantation, implantation, decidual function and placental growth.

**Interleukin-1 (IL-1):** It seems to be the first cytokine active in the embryo-endometrial crosstalk, which then results in a second wave of cytokines. It is not yet proven that missing IL-1 receptor in the endometrium has a detrimental effect on the cytokine cascade system, which is essential for implantation. Adding IL-1 to embryo culture media may improve implantation but this is not yet clinically tested.

**Integrins**

Cell adhesion molecules fall into four major groups: Integrins, cadherins, selections, and other 1 gm super family. Integrins are present in the endometrium throughout the menstrual cycle and the expression of integrins are hormonally regulated. Several studies have shown that these may be potential markers for endometrial receptivity. Insufficient integrin expression is seen in many conditions with infertility like luteal phase insufficiency, endometriosis, unexplained infertility, and presence of hydrosalpinx.

The immunohistochemical express of $\alpha_2$ in midluteal endometrial samples seems to be a marker of endometrial receptivity.

**Glycodelin**: It is one of the most abundant products of glandular cells in the late secretary endometrium. It has been proposed as the most reliable non-invasive master of endometrial function in women.

Measurement of glycodelin in the uterine flushing fluid may be more informative compared to plasma levels in the evaluation of endometrial receptivity. Glycodelin measurement in uterine flushing is less harmful and less invasive than taking biopsy.

**MUC 1**: Change in MUC 1 glycoforms also seem to correlate with the receptive window. MUC 1 may facilitate embryo adhesion to the endometrium. In support of this hypothesis, studies in mice have suggested that histo-blood group-related carbohydrate antigens play a direct role in the implantation process. The clinical value of MUC1 as a marker of uterine receptivity is uncertain at present. Detailed extended studies are going on and results are awaited.

This discussion reviews, the current knowledge and status of the biochemical factors implicated in the unimplantation process. These factors are produced, expressed and secreted by the endometrium and regulated by hormones.

Endometrium has receptors for reception by hormones, which have to reach the endometrium via the spiral network from the uterine artery. Blood supply to the endometrium is also governed by the hormones and cyclical changes in the endometrial blood flow, and the main uterine blood flow have been well documented by color Doppler during the various phases of menstrual cycle.

It has further been well established that a decreased blood flow in the uterine and its vascular branchings is a course of infertility probably due to implantation failure and poor endometrial receptivity.

Because biochemical evaluation of uterine receptivity is difficult to perform in the daily clinical routine and daily IVF setups, an easier, reproducible and accurate marker for endometrial receptivity was postulated to be the transvaginal ultrasound study of endometrium and its blood supply by color Doppler.

Various studies described the receptive endometrium as the one, which has an endometrial thickness of more than 7 mm, 3 layered, five-layered, etc.

**ROLE OF ULTRASOUND AND COLOR DOPPLER**

Ultrasound (TVS) offers a simple, reliable, reproducible, quick and non-invasive method for assessing the female pelvis.

**Endometrial Changes**

1. Endometrial growth in follicular phase tortuous.
2. Increase in glands but quiescent till ovulation.
3. At ovulation, the glands become tortuous and formation of spiral arteries occurs.
4. During implantation time (days 20, 21 and 22), the endometrium is thick, edematous and contains predecidual cells. Epithelias lining has a mucus film and microvilli and pinopodes. These are not present at day 16 and vanish by day 24 in both spontaneous or induced cycles. These changes and pinopodes are said to help in the process of anchoring and sustaining the embryo.

5. Extensive biochemical changes also occur in the endometrium, which are not fully understood and involve synthesis of various proteins, salicylic acid, 3 fucosyl-N acetyl lactosamine, electro-negative, etc. Retinoic acid (RAR) evolves all these changes and these are deficient in primary infertility cases.

6. Electrocharges, mitotic changes, molecular and biological changes are also activated following ovulation. The endometrial changes at all stages are regulated by steroids.

**Ultrasound Technique for Uterine Biophysical Profile**

To perform the UBP, special care should be taken. The following guidelines are recommended (Applebaum 96):

1. To determine the presence of a five-line appearance, information from both the transabdominal and transvaginal studies may be useful. For example, although a five-line appearance may be noted transabdominally, it may not always be possible to see it endovaginally due to uterine position (and vice versa). In this case, a five-line appearance is considered to be present and endometrial vascular penetration may be estimated when performing the endovaginal study.

2. Perform the Doppler study slowly. The flow of blood in the endometrium is of low velocity; it may take time for the ultrasound machine to register the presence of blood flow and create the image. If one sweeps through the endometrium too quickly, flow may not be seen. Additionally, endometrial blood flow has a mercurial personality—it may appear as if it comes and goes. It may also appear in some areas and not in others. Do not observe hastily.

3. Endeavor to make the endometrium as specular reflector as possible. Use the techniques of manual manipulation of the anatomy and probe pressure to achieve this.

4. Scan endovaginally both coronally and sagittally. There may be a difference in how well the blood flow is imaged.

5. When measuring the endometrium in the AP dimension, try to obtain the value when no contraction affecting it is present. Contractions may affect this value. Also when possible, obtain the measurement in a standard plan, such as when both the endometrial and cervical canals appear continuous.

**Uterus**

The location of uterus is in the lesser pelvis between the urinary bladder and rectum. Although, usually a midline structure, lateral deviations of the uterus are not uncommon.
systolic spectral flow pattern with a mean PI of 5.2 ± 0.4. Evaluations, performed during treatment and on days 13 and 14, showed a spectral tracing that was broader with an uninterrupted diastolic component. The mean PI was 1.5 ± 0.2. On days 26 and 27, no significant differences were noted (mean PI = 1.7 ± 0.3).

The possibility that decreased uterine blood flow may be associated with infertility was investigated by Goswamy et al. In their study, the uterine arteries of patients who had been unsuccessful in three attempts at IVF were interrogated using Doppler ultrasound. Almost half demonstrated a poor mid-secretary uterine response. Of these, patients demonstrating improved uterine perfusion on oral hormone therapy had a pregnancy rate comparable to or better than that obtained in the first three attempts by other patients. Although the number of patients in each group studied were too small for statistical analyses, the trend suggested that improving uterine perfusion may improve the outcome of IVF therapy. Two years later, results from a greater number of patients were reported. This confirmed the results of earlier work. The data indicated that 20 percent of all women undergoing IVF therapy had poor uterine perfusion. This latter work utilized the concept of a perfusion index to analyze the flow velocity waveform. This index is derived from the ratio of the area under the curve of the systolic component of the flow velocity waveform (S) over the area beneath the diastolic component (D) (Perfusion Index = S/D). This analysis allows for a different approach for the evaluation of waveform.

**Endometrium**

Sonographically, the endometrium is one of the most dynamic structures in the body. During the reproductive years of a normal female, the uterus undergoes sonographically detectable alterations characterized by cyclical changes in the echo pattern of the endometrium. In fact, it is possible to infer the approximate day of a normal woman’s menstrual cycle by the sonographic appearance of the endometrium.

From the first day of the menstrual cycle until the mid-cycle, the normal endometrium progressively thickens and develops sonographically detectable strata. This appearance can be described as layered, trilaminar or five-line (term of preference). Past the mid-cycle, the normal endometrium brightens and progressively thins. These sonographic endometrial patterns appear to be related to the changes in the glandular and vascular elements of the endometrium during the menstrual cycle.

Fischer et al determined that the endometrium is thickest during the secretory phase (3.6 ± 1.4 mm), less thick during the proliferative phase (2.9 ± 1.0 mm) and thinnest during menstruation.4,14,15 These values are for the half-thickness as measured from the endometrial canal to the endometrial-myometrial junction. Full thickness measurements ranged from 4 to 12 mm with an average thickness of 7.5 mm.

The endometrium will either slough if no pregnancy occurs or will undergo various changes in the event of pregnancy.

The blood supply to the endometrium is derived from branches of the uterine arteries. Emanating from the arcuate arteries (vide supra) are the radial arteries. These vessels run through the myometrium to just outside the endometrium where they form terminal branches of two types straight and coiled. The straight branches, also known as the basal arteries, supply the basalis layer of the endometrium. The coiled branches, also known as the spiral arteries, traverse the endometrium and supply the functionalis layer. The spiral arteries like the endometrium and unlike the basal arteries are responsive to the hormonal changes of the menstrual cycle.

In preparation for implantation, the endometrium undergoes transformations influenced by the ovarian hormones produced during the early secretory phase. These modifications include increase in the rate of blood flow, number of cells populating the stroma and epithelium, uterine oxygen consumption, oxygen diffusion into the uterine lumen and a generalized edema.

The spiral arteries respond to the hormonal changes of the menstrual cycle and undergo transformations as well. These responses include proliferation of the endothelium, thickening of the wall and coiling. These vessels play an important role in implantation. The chances for a normal implantation may be reduced if the spiral arterioles are inadequately developed.

Changes in the endometrial vascularity appear on color Doppler examination, which may reflect the histologic changes described by the pathologists. Some investigators appear unable to demonstrate this. Perhaps, this is due to equipment and/or technique differences.

If one divides the endometrial and periendometrial areas into the following four zones:

- **Zone 1**: A 2 mm thick area surrounding the hyperechoic outer layer of the endometrium.
- **Zone 2**: The hyperechoic outer layer of the endometrium.
- **Zone 3**: The hypoechoic inner layer of the endometrium.
- **Zone 4**: The endometrial cavity.

It is possible to see variations in the depth of vascular penetration before, during and after the mid-cycle. Based upon observations, most patients without diagnosed infertility (presumed normal) usually demonstrate flow into zone 3 by the mid-cycle.

Doppler ultrasound has been used as a method to predict a negative outcome for a given IVF cycle pretransfer. If failure could be predicted, the embryos could be frozen until a more favorable cycle occurs. This could prevent embryo wastage and subsequent patient disappointment.

Sterzik et al examined the ovarian and uterine arteries on the day of follicle aspiration. The conclusion at which they arrived was that in patients who became pregnant after embryo transfer, the RI of the uterine arteries were significantly lower than those who did not get pregnant.
Steer et al demonstrated that patients with a low uterine artery PI on the day of embryo transfer were more likely to conceive than those with a high PI. In this series, no one with a PI > 3.0 conceived.

Battaglia et al demonstrated a progressive decrease in the PI of the uterine arteries during the second half of the menstrual cycle in successful IVF pregnancies. In using color Doppler technique, inadequate vascular penetration of endometrial blood flow (not within zone 3) prior to transfer has been associated with an unfavorable outcome. Vascular penetration towards the endometrial canal differs among patients. In patients with uterine artery PI of less than 3.0, thus far, my results have not revealed any successful pregnancies in IVF patients unless there is vascularity demonstrated either within zone 3 or within zones 3 and 4 prior to transfer. Successful pregnancies with demonstrable blood flow in zone 4, suggesting the presence of an intracavitary mass, have been noted. Subsequent investigations have confirmed the validity of this finding in patients undergoing non-IVF stimulation cycles.

These color Doppler findings in unsuccessful cycles may relate to the histologic findings described by Sterzik et al. In their study of 58 IVF patients, majority demonstrated an immature endometrium at the time of embryo transfer. The abnormalities included a variety of patterns, all indicating a lack of secretory transformation suggesting unpreparedness for implantation.

The complete evaluation of the IVF patient may require attention to the gray-scale appearance of the endometrium as well. Glissant et al noted that the thickness of the endometrium was significantly greater in cycles resulting in a pregnancy than those which did not, however it was not possible to predict the probability of a pregnancy based upon endometrial thickness. In contrast, Welker et al were unable to relate endometrial thickness to outcome, but were able to relate endometrial pattern to outcome. In their experience, the five-line appearance was most likely to be associated with implantation. Smith et al felt that both endometrial thickness and pattern were important. Other investigators have also looked at the relationship between endometrial thickness or texture and outcomes.

In a retrospective study of non-IVF medically stimulated cycles, Kepic et al determined that endometrial thickness and pattern, follicle size and estradiol levels correlated not only with the likelihood of pregnancy, but also with subsequent outcome (i.e. miscarriage vs nonmiscarriage).

In the event of pregnancy, low resistance flow to the uterus remains. The finding of blood flow within the endometrium, on gray-scale examination, has been reliably associated with the gravid state in both IVF/infertility and non-IVF patients. This flow has been visible as early as day 27 after the last normal menstrual cycle prior to visualization of the gestational sac and with a beta-hCG of 156. The distribution of this finding may be either local or general. This is similar to the pathologic specimens in which endometrial changes induced by the sex hormone demonstrate non-uniform, regional differences.

In one case, very localized blood flow changes were demonstrated on gray-scale examination. It was in this area that the gestational sac eventually appeared. Histologically, at the time of implantation (the seventh day following ovulation), hypertrophic and proliferative changes of the spiral arterioles occur within the endometrium. As the blastocyst implants over these spiral vessels, those beneath the lower pole of the implanting blastocyst hypertrophy further with the capillaries in the surrounding stroma dilating widely and their walls thinning. It is possible that these are the changes that were reflected on ultrasound examination. The finding of endometrial blood flow in not specific for intrauterine gestations, as it can be seen in the presence of ectopic pregnancies. Possibly, this represents a sonographic appearance of the Arias-Stella reaction. It can also be seen with endometrial hyperplasia.

**Uterine Biophysical Profile**

Certain sonographic qualities of the uterus are noted during the normal mid-cycle. These include:
1. Endometrial thickness in greatest AP dimension of 7 mm or greater (full-thickness measurement).
2. A layered (five-line) appearance to the endometrium.
3. Blood flow within zone 3 using color Doppler technique.
4. Myometrial contractions causing a wave like motion of the endometrium.
5. Uterine artery blood flow, as measured by PI, less than 3.0.
7. Myometrial blood flow seen on gray-scale examination (internal to the arcuate vessels).

The uterine scoring system for reproduction (USSR) comprises evaluation of the following parameters:
1. Endometrial thickness (full-thickness measured from the myometrial-endometrial junction to the endometrialm-yoimetrial junction)
2. Endometrial layering (i.e. a five-line appearance)
3. Myometrial contractions seen as endometrial motion
4. Myometrial echogenicity
5. Uterine artery Doppler flow evaluation
6. Endometrial blood flow
7. Gray-scale myometrial blood flow.

Each parameter is scored as follows:

1. Endometrial thickness
   - a. mm = 0
   - b. 7-9 mm = 2
   - c. 10-14 mm = 3
   - d. > 14 mm = 1

2. Endometrial layering
   - a. No layering = 0
   - b. Hazy five-line appearance = 1
   - c. Distinct five-line appearance = 3
3. Myometrial contractions (seen as wave-like endometrial motion high-speed playback from videotape)
   a. 3 contractions in 2 minutes (real-time) = 0
   b. 3 contractions in 2 minutes (real-time) = 3

4. Myometrial echogenicity
   a. Coarse/inhomogeneous echogenicity = 1
   b. Relatively homogeneous echogenicity = 2

5. Uterine artery Doppler flow
   a. PI–3.0 = 0
   b. PI–2.99 = 0
   c. PI–2.49 = 1
   d. PI < 2 = 2

6. Endometrial blood flow within zone 3
   a. Absent = 0
   b. Present, but sparse = 2
   c. Present multifocally = 5

7. Myometrial blood flow internal to the arcuate vessels seen on gray-scale examination
   a. Absent = 0
   b. Present = 2

The values assume a technically adequate ultrasound examination with no abnormalities of uterine shape or development, no other gross uterine abnormalities, e.g. significant masses and a normal ovarian cycle, e.g. without evidence of ovarian-uterine dyscoordination). A male factor component to the infertility is not present.

In limited experience (Applebaum) with this system thus far, a USSR “perfect score” of 20 has been associated with conception 100 percent of the time. The number of patients in which we predicted successful conception cycles based upon the UBP and USSR perfect score was 5. This group included 2 spontaneous cycles (non-IVF, non-IUI), two IUI and one IVF. Scores of 17 to 19 (10 patients) have been associated with conception 80 percent of the time. Scores of 14 to 16 (10 patients) have a 60 percent chance, while scores of 13 or less (25 patients) have resulted in no pregnancies.

Absent endometrial flow, despite highest values for the other parameters, has always been associated with no conception.

Our initial observations were based upon experiences with both “normal” non-infertility patients and patients treated for infertility. The observations were categorized and then applied as a system to patients with diagnosed infertility all comers. I did not divide the patient population into subgroups based upon treatment protocol, age, cause, etc.

THE EXPERIENCE AT MALHOTRA TEST TUBE BABY CENTER, AGRA

With a perfect USSR score of 20.97 percent conception was seen: We had 22 spontaneous cycles (non-IUI, non-IVF), 15 IUI cycles and 5 IVF cycles. Total 42 cases: 41 conceptions.

1. Scores of 17 to 19 (20 patients), conception rate of 84 percent.
2. Score of 14 to 16 (20 patients), conception of 60 percent.
3. Score 13 and less (20 patients), only one pregnancy.

No doubt, other factors apart from sonographic signs of “uterine receptivity” are at work in determining conception. We attempted control for all factors, which we could detect sonographically. Factors such as scoring of either embryos prior to transfer or ova at the time of aspiration, as some of our laboratories do, were not considered. We are sure that the quality of the transfers is important.

Our results are preliminary and substantially more patients need to be evaluated. We have no illusions that the parameters and scoring numbers will remain the same and we suspect that the cut-off values and success rates will evolve over time, especially in the hands of other investigators. We suspect that the quality of the laboratory affects the values but have not yet investigated this. We also do not believe that any group of findings will work perfectly 100 percent of the time. Nonetheless, we do believe that there is a “normal” appearance to the “normal” mid-cycle which is ascertainable, the recognition of which can be applied to the benefit of our infertile patients.

At Malhotra Test Tube Baby Center, Applebaum criteria was applied and following results were obtained.

With a perfect USSR Score of 20.97 percent conception was seen: we had 222 spontaneous cycles (non-IUI, non-IVF), 21 IUI cycles and 62 IVF cycles. Total 80 cases of perfect score of 20: 54 conceptions.

1. Scores of 17 to 19, conception rate of 79 percent (Spontaneous) 60 percent in IUI cycles and 0 percent IVF.
2. Score of 14 to 16, conception of 44 percent in spontaneous 54 percent IUI cycle and 20 percent in IVF.
3. Score 13 and less, only one pregnancy in natural spontaneous and 1 in IUI group.

Experience at Malhotra Test Tube Baby Center, Agra (2000)

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IDEAL ENDO METRIAL RECEPTIVITY TESTS

Endometrial preparations for implantation is a very intricate and complex mechanism involving hormonal stimulation of receptors, endometrial cell response, biochemical and immunological cellular response, proper spiral artery blood flows, good embryos, and embryo-endometrial interaction. These tests are invasive and expensive, cannot be repeated many times and need highly intricate laboratory and trained personal. What we have with us today is a simple, reliable, accurate, reproducible, inexpensive test in the form of transvaginal ultrasound analysis of the endometrium and blood supply. Various scoring systems provide a fairly accurate assessment of the endometrial receptivity.

Most investigators agree that a high degree of endometrial perfusion shown by color or by power Doppler indicates a more receptive endometrium. The consensus on how to do ideal endometrial blood flow study and endometrial receptivity scoring still tails (Applebaum, and child et al., Kupesic Salle).

CONCLUSION

Transvaginal color and power Doppler, and 3D PD evaluation of endometrial and subendometrial blood flow distribution is a simple and effective method to evaluate endometrial receptivity. Endometrial and subendometrial blood flow is indicative of good endometrial receptivity.

REFERENCES


