10.5005/jp-journals-10006-1481

EDITORIAL

Endometrial Receptivity and Scoring for Prediction of Implantation and Newer Markers

[1]Neharika Malhotra, [2]Jaideep Malhotra, [3]Amreen Singh, [4]Pallavi Gupta, [5]Narendra Malhotra

How to cite this article: Malhotra N, Malhotra J, Singh A, Gupta P, Malhotra N. Endometrial Receptivity and Scoring for Prediction of Implantation and Newer Markers. J South Asian Feder Obst Gynae 2017;9(2):143-154.

Source of support: Nil

Conflict of interest: None

Date of received: 10 January 2017

Date of acceptance: 10 March 2017

Date of publication: April 2017

INTRODUCTION

Assisted reproductive technologies (ARTs) are here to stay. At 11.30 am, 25 July, 1978, with the birth of Louise Brown, infertility was conquered by *in vitro* fertilization (IVF) and embryo transfer. In the last 20 years, we have had over 30,000 IVF babies from over 500 IVF centers. In India, around 20 IVF centers contributed to about 5,000 pregnancies using this technology. With improvement in stimulation protocols, better understanding of the physiology and pharmacology of gonadotropins, and with the advent of recombinant follicle stimulating hormone, better monitoring facilities, state-of-the-art lab equipment and facilities for intracytoplasmic sperm injection (ICSI), testicular sperm aspiration, and microsurgical epididymal sperm aspiration 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 the physiology of ovaries, the uterus, and endometrium during various phases of normal menstrual cycles, ovulation induction, super ovulation, and controlled ovarian hyper-stimulation as well as implantation and normal pregnancy.

Based on numerous studies from 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 that eludes a scientific basis and high success rates; therefore, to overcome this, various centers are increasing the number of embryos being transferred, which often leads to multiple gestations, painful embryo reductions, and spontaneous early pregnancy loss; hence, it becomes an absolute necessity to evaluate the uterus and the endometrium by color Doppler imaging, so that we can perform embryo transfers in only favorable uteri. The fertilized embryos not used can be frozen for use in a favorable cycle based on the various and scoring criteria like uterine biophysical profile (UBP) and uterine scoring system for reproduction.[1,2]

[1-5]Department of Rainbow IVF, Rainbow Hospitals, Agra, Uttar Pradesh, India

Corresponding Author:

PHYSIOLOGY OF IMPLANTATION

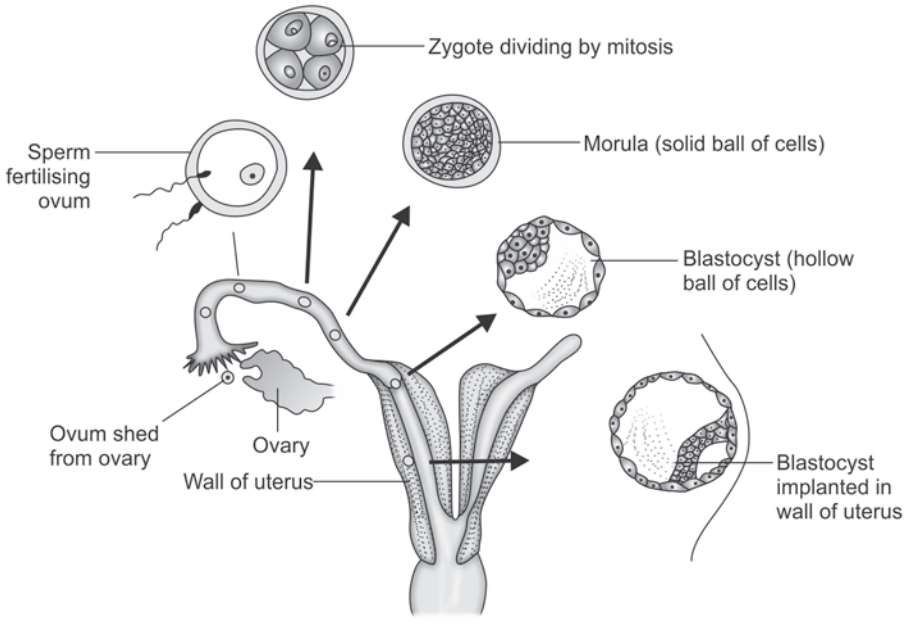
Implantation presents the greatest challenge to the ART specialist, in whichever form it is applied, for it is a known fact that less than one-third of human embryos placed in the uterus complete implantation. The uterus also prepares for implantation along with the hatching blastocyst. The endometrium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

- Attachment of blastocyst to the endometrium.
- Penetration of blastocyst into the stroma.

  The stroma will help by:
- Anchoring
- Sustaining the embryo
- Control the invasiveness of trophoblast
- Production of growth factors and substrates
- Stroma responds to the proteins and hormones of ovary and embryo.

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

Implantation is an important step in establishing pregnancy and still the scientific basis for the failure of implantation eludes us. Implantation is difficult to study because our knowledge of the occurrences during the first week of human life *in vivo* is very limited. 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 and (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 these have been based on:

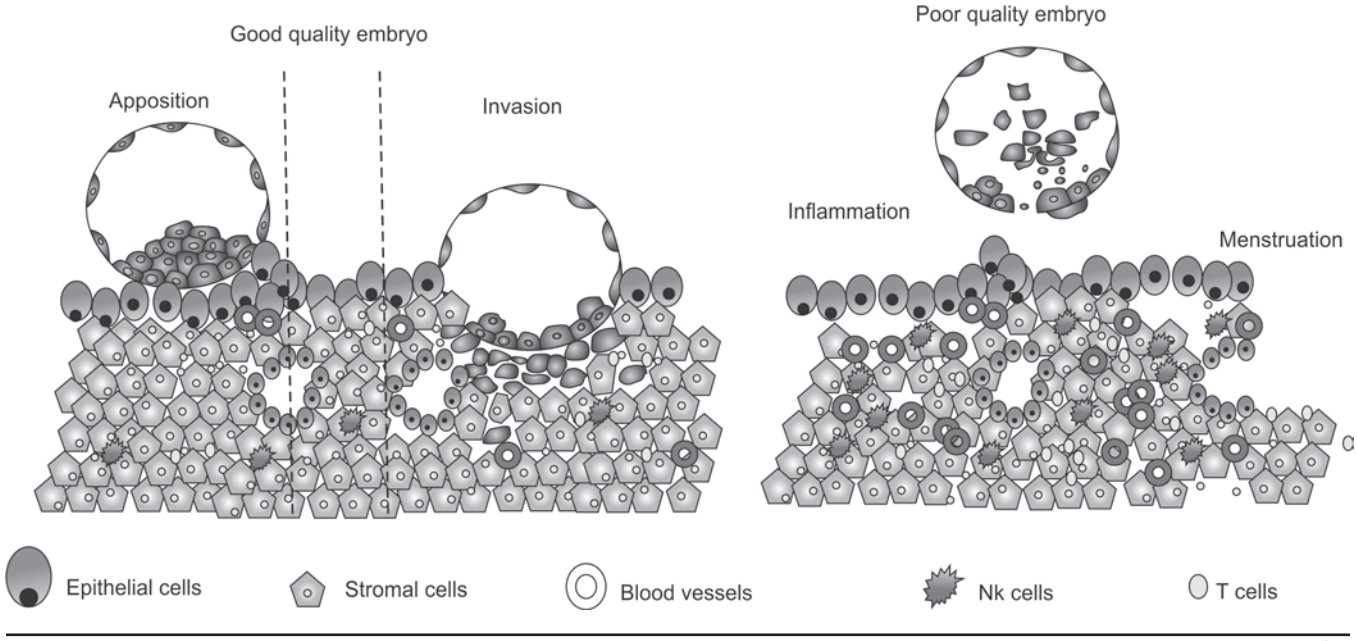
- Biochemical evaluation
- Ultrastructure study of pinopodes
- Ultrasound scoring systems
- Ultrasound assessment of subendometrial blood flow.

BIOCHEMICAL EVALUATION AND MARKERS[7]

- Cytokines
  - Leukemia inhibitory factor (LIF)
  - Colony stimulatory factor (CSF)
  - Interleukin-1 (IL-1)
- (b) Integrins
  - Glycodelin
  - Mucin 1 (MUC 1)

Many biochemical factors, which are 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

The LIF, interleukin 1, and colony stimulatory factor-1 are actively produced by the endometrium cells, and they are shown to be important in the cross-talk between embryo and endometrium.

Leukemia inhibitory factor is likely to influence pre-implantation, implantation, embryo development, and placentation. The LIF can be screened in endometrial flushings and should prove to be a screening marker for endometrial receptivity.[8]

Colony stimulatory factor-1: Low levels of CSF-1 have been associated with recurrent, spontaneous abortions. Increased production of CSF-1 is expressed throughout pre-implantation, implantation, decidual functions, and placental growth.[9]

Interleukin-1 seems to be the first cytokine active in the embryo–endometrial cross-talk, which then results in a second wave of cytokines. It is not yet proven that missing IL-1 receptors 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.[10]

Integrins: (Glycodelin, MUC 1)

Cell adhesion molecules fall into four major groups: Integrins, cadherins, selectins, and the other l g super family. Integrins are present in the endometrium throughout the menstruation cycle, and the expression of integrins is hormonally regulated. Several studies have shown that these may be potential markers for endometrial receptivity. Insufficient integrin expression is seen in many conditions associated with infertility, Such as luteal phase insufficiency,[11] endometriosis,[12] unexplained infertility,[13] and presence of hydrosalpinx.

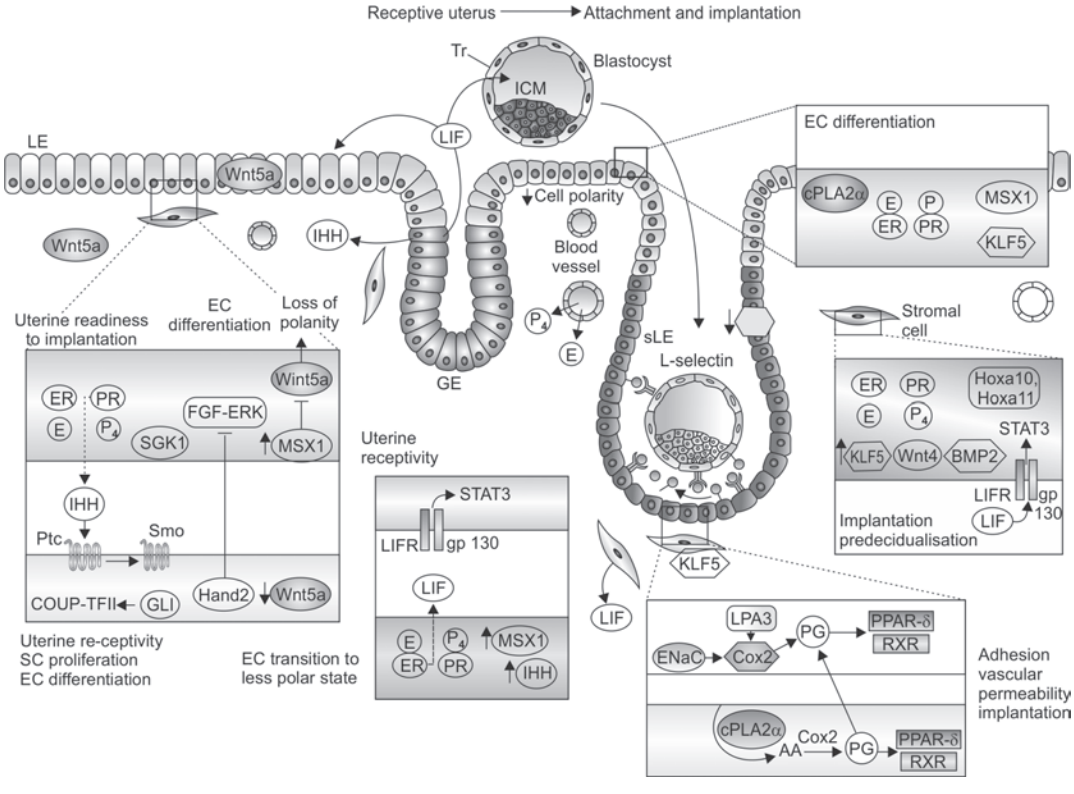
The immunohistochemical expression of α_v β_3 in midluteal endometrial samples seems to be a marker of endometrial receptivity.

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

Measurement of glycodelin in the uterine flushing fluid may be highly informative compared with plasma levels during evaluation of endometrial receptivity. Glycodelin measurement in uterine flushing is less harmful and less invasive than taking a biopsy.[14]

Mucin 1: Changes in MUC 1 glycoforms also seem to correlate with the receptive window. The MUC 1 may facilitate embryo adhesion to the endometrium.[15] In support of this hypothesis, studies in mice have suggested that histo-blood group-related carbohydrated antigens play a direct role in the implantation process.[16] The clinical value of MUC 1 as a marker of uterine receptivity is at present uncertain. Detailed studies are going on, and the results are awaited.

This paper discusses and 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.[17]



The endometrium exhibits reception, as it has receptors for 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.[18-20]

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.[21]

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.[22-25]

Various studies described the receptive endometrium as one that has an endometrial thickness of more than 7 mm, is three layered or five-layered, etc.[26]

ROLE OF ULTRASOUND AND COLOR DOPPLER

Ultrasound (TVS) offers a simple, reliable, reproducible, quick, and noninvasive method for assessing the female pelvis.[26]

Endometrial Changes

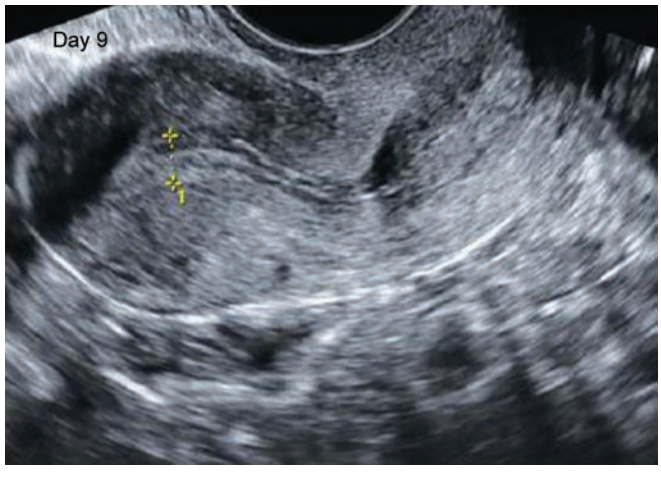
- Endometrial growth in follicular phase.
- Increase in glands, but they are quiescent until ovulation.
- At ovulation, the glands become tortuous with the formation of spiral arteries occur.
- During implantation time (day 20, 21, and 22), the endometrium is thick, edematous, and contains predecidual cells. Epithelial 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 the presence of pinopodes are said to help in the process of anchoring and sustaining the embryo.
- Extensive biochemical changes also occur in the endometrium, which are not fully understood; they involve synthesis of various proteins, salicylic acid, 3-fucosyl-N acetyl lactosamine, maintenance of electronegative status, etc. Retinoic a (RAR) are involved in all these changes and these are deficient in primary infertility cases.
- Electro charges, mitotic changes, and molecular biological changes are also activated following ovulation. The endometrial changes at all stages are regulated by steroids.

Ultrasound Technique for UBP

To perform the UBP, special care should be taken.

The following guidelines are recommended (Applebaum 96).

- To determine the presence of a 5-line appearance, information from both the transabdominal and transvaginal studies may be useful. For example, although a 5-line appearance may be noted transabdominally, it may not always be possible to see it endovaginally due to the uterine position (and vice versa). In this case, a 5-line appearance is considered to be present, and endometrial vascular penetration may be estimated when performing the endovaginal study.
- Perform the Doppler study slowly. The flow of blood in the endometrium is of low velocity; thus, 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 many 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.
- Endeavor to make the endometrium as a specular reflector as possible. Use the techniques of manual manipulation of the anatomy and probe pressure to achieve this.
- Scan endovaginally both coronally and sagittally. There may be a difference in how well the blood flow is imaged.
- When measuring the endometrium in the anterioposterior dimension, try to obtain the value when no contraction is influencing it. 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

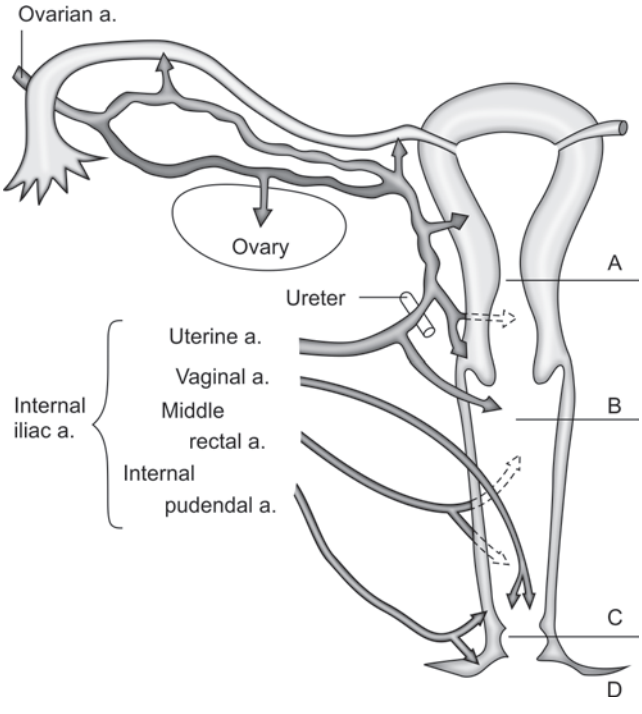
The location of the uterus is in the lesser pelvis between the urinary bladder and the rectum. Although usually a midline structure, lateral deviations of the uterus are not uncommon.

The broad ligaments extend from the uterus laterally to the pelvic sidewalls. The broad ligaments contain the fallopian tubes and vessels. The uterosacral ligaments function to keep the uterus in an anterior position. They arise from the upper cervix posteriorly and extend to the fascia over the second and third sacral vertebrae. The round ligaments arise anterior to and below the fallopian tubes and cross the inguinal canal to end in the upper part of the labia majora.

The normal adult uterus measures approximately 7.0 to 9.0 cm in length, 4.5 to 6.0 cm in width, and 2.5 to 3.5 cm in depth (anterio-posterior dimension). The cervix-to-corpus ratio in adults is 1:2.

The blood supply to the uterus is from the uterine artery, which is a branch of the internal iliac artery. The uterine artery enters the uterus at the cervico-corporal junction and ascends along the lateral aspect of the uterine body to the cornua. At the uterine cornua, an adnexal branch arises, which supplies the ipsilateral ovary and anastomoses with the ipsilateral ovarian artery.

From the uterine artery originate perforating branches. These extend through the serosa. The uterine arteries anastomose via the anterior and posterior arcuate vessels. These vessels are located at the junction of the outer and middle thirds of the myometrium, between the exterior longitudinal muscle fibers and the inner oblique muscle fibers.



During the reproductive years, cyclical changes in uterine blood flow can be demonstrated using both color and duplex Doppler techniques.

The usual pattern of uterine blood flow throughout the menstrual cycle is that perfusion increases in response to rising plasma estrogen and progesterone, and decreases with the periovulatory fall in estrogen. Although the experience of others may differ, some investigators have found that the lowest PI values are obtained around days 8 and 21, while the highest values are obtained around days 1, 7, 14, and 17. Significant changes in diastolic blood flow at the different times of the cycle may not be noted. Generally, the index values for the uterine artery ipsilateral to the ovary containing the dominant follicle are lower than the contralateral artery.

Other patterns of uterine artery blood flow have been described. When the uterine arteries were interrogated at the level of the uterine cornua, the PI reached its peak by day 11 and remained relatively constant until day 16. The lowest values were generally obtained around days 1 and 21. At the level of the cornua, end-diastolic flow was frequently absent during the early follicular phase, but was demonstrable by the luteal phase.

These cyclical changes reflected in the low-velocity waveforms and index values appear to be mediated by the reproductive hormones. Patients with inactive ovaries and receiving transdermal estradiol and vaginal progesterone therapy were studied using transvaginal ultrasound technique. These patients received their medications on a 28-day regimen. The pre-treatment (baseline) valuation demonstrated a narrow systolic spectral flow pattern with a mean PI of 5.2 ± 0.4 Valuations performed during treatment and on days 13 to 14 showed a spectral tracing that was broader with an uninterrupted diastolic component. The mean PI was 1.5 ± 0.2. On days 26 to 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 for three attempts during 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 with or better than that obtained in the first three attempts by other patients. Although the number of patients in each group studied was 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 the earlier work. The data indicated that 20% of all women undergoing IVF therapy would have poor uterine perfusion. This latter work utilized the concept of a PI to analyze the flow velocity waveform. This index is derived from the ratio of the area under the curve of the systolic component constituting the flow velocity waveform (*S) over the area beneath the diastolic component (*D) (Perfusion Index = *S/*D). This analysis allows for a different approach to the valuation of the waveform.

Endometrium

Endometrium is the innermost lining of the uterus. It has receptors that respond to the cyclic patterns of hormones like estrogen and progesterone, and to a complex interplay among its own autocrine and paracrine factors. It is the most crucial structure to be evaluated in an IVF cycle.

Sonographically, the endometrium is one of the most dynamic structures in the body. During the reproductive years of a normal female, the uterus undergoes ultrasonographically 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.

Endometrium in the Menstrual Cycle

The effects of varying concentrations of estrogen and progesterone throughout the course of the menstrual cycle have characteristic effects on the endometrium.

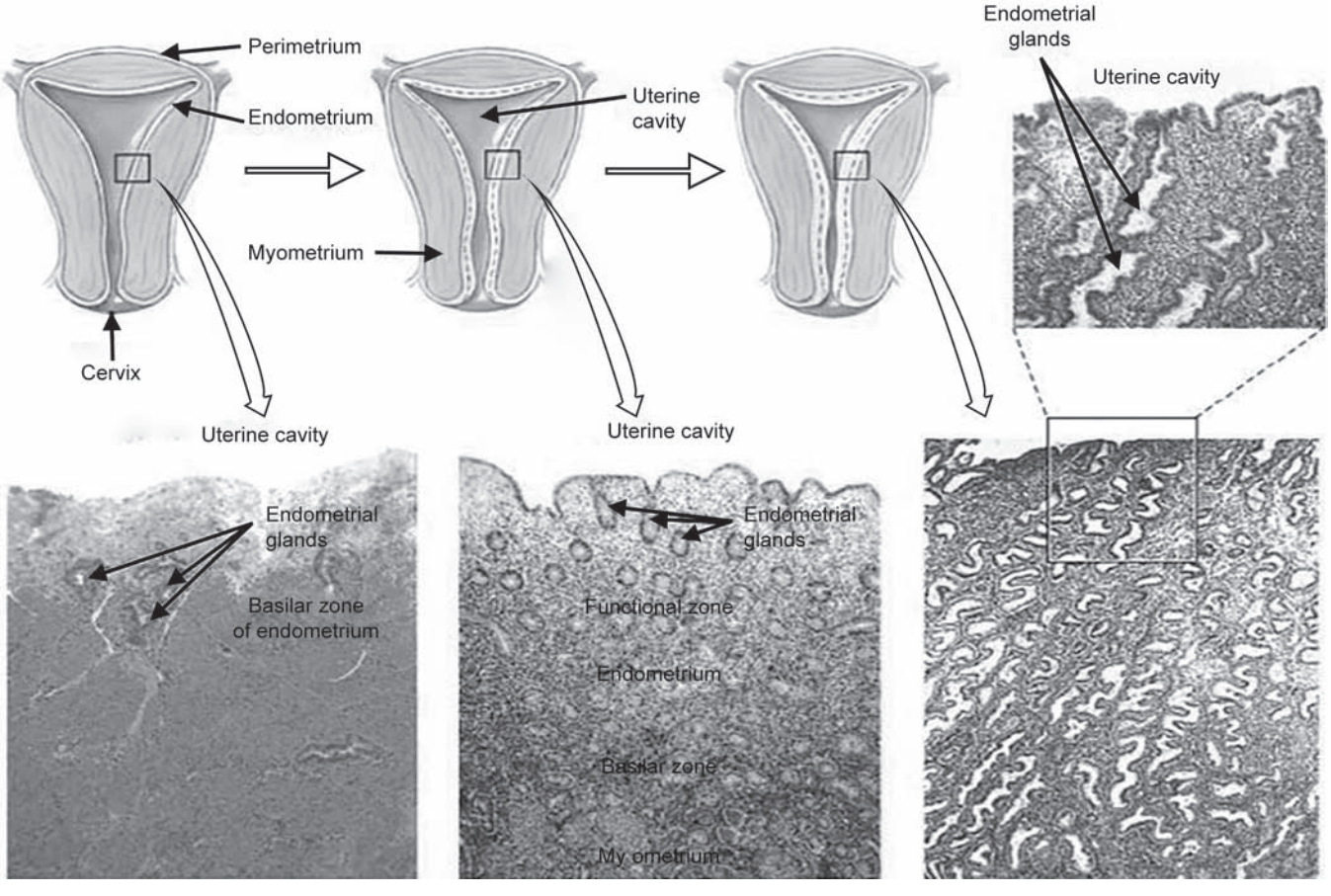
The endometrial cycle consists of three phases.

1. The proliferative phase
2. The secretory phase
3. Menstrual phase

The proliferative phase coincides with the midfollicular to late follicular phase of the menstrual cycle. Under the influence of the rising plasma estradiol concentration, the stromal and epithelial layers of the uterine endometrium undergo hyperplasia and hypertrophy and increase in size and thickness.

From the first day of the menstrual cycle till the midcycle, the normal endometrium progressively thickens and develops sonographically detectable strata. This appearance can be described as layered, trilaminar or 5-line (term of preference).

The secretory phase begins on the day of ovulation and coincides with the early to midluteal phase of the menstrual cycle. The endometrium contains numerous progesterone receptors. Under the combined action of progesterone and estrogen, the endometrial glands become coiled, store glycogen. The stroma increases in vascularity and becomes edematous, and the spiral arteries become tortuous.



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.

In the Menstrual phase necrotic changes and abundant apoptosis occur in the secretory epithelium as it collapses. The arteries constrict, reducing the blood supply to the superficial endometrium. Leukocytes and macrophages invade the stroma and begin to phagocytose the ischemic tissue. Leukocytes persist in large numbers throughout menstruation, providing resistance against infection to the denuded endometrial surface

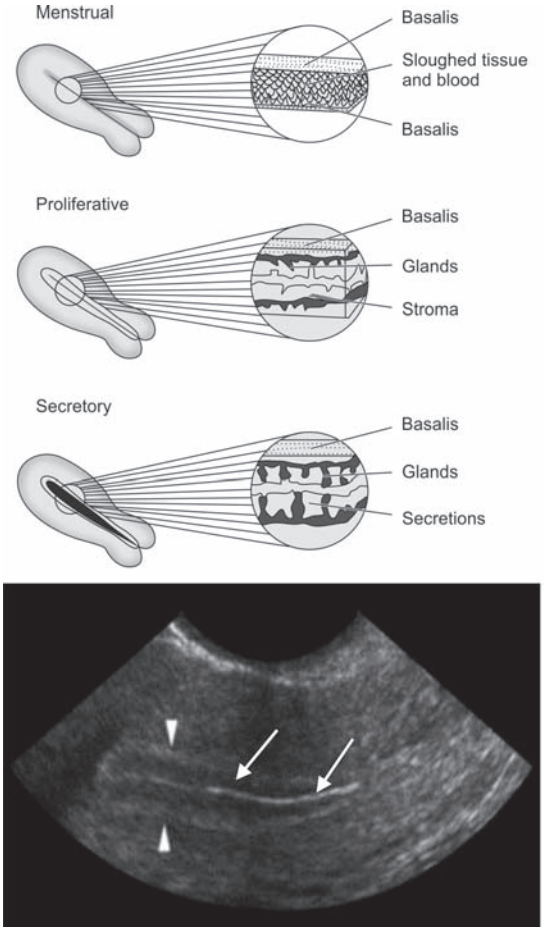
Fischer et al determined that the endometrium is thickest during the secretory phase (3.6 ± 1.4 mm), less thick during the proliferative phase (2.9 ± 1.0 mm) and thinnest during menstruation 14 to 15. These values are for the half-thickness as measured from the endometrial canal to the endometrial-myometrial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

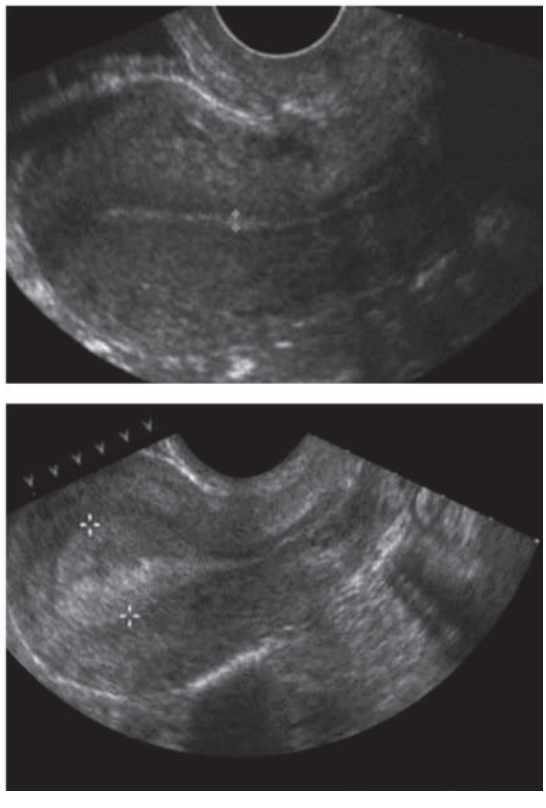
The endometrium will either slough if no pregnancy occurs or will undergo various changes in the event of a pregnancy.

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.

ENDOMETRIAL ASSESSMENT

Ultrasound Assessment of the endometrium is a standard procedure during the diagnostic workup and treatment of infertility. The effects of varying concentrations of estrogen and progesterone throughout the course of the menstrual cycle have characteristic effects on the endometrium. The endometrial changes that occur can be visualized with sonography. Transvaginal ultrasonography is often used to examine various parameters of endometrium like endometrial thickness, morphology and blood flow status to predict uterine receptivity.[3,27] Endometrium pattern, endometrium thickness, and end-diastolic blood flow were shown to be the most effective combination for evaluation of uterine receptivity.[4] (Dechaud H)





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

Endometrial Thickness

Endometrial thickness is defined as the maximal distance between the echogenic interfaces of the myometrium and the endometrium, measured in the plane through the central longitudinal axis of the uterus. In IVF stimulated cycles, the endometrium increases 1.9 mm between days 7 and 9 of the stimulation treatment, 0.9 mm between days 9 and 11 and 0.6 mm between the latter and the day of hCG administration.[5] Endometrial thickness in greatest AP dimension of 7 mm or greater is associated with higher pregnancy rate.

Endometrial Pattern

Endometrial pattern is relative echogenisity of endometrium and myometrium as seen on longitudinal transvaginal scan.There are various scoring systems being used for garding endometrium

Smith et al classifies the endometrium into four types according to the echotexture pattern. They are considered to be useful in deciding on receptivity in IVF.The Smith system is as follows[6,25]

- *Grade I*: Hyperechoic
- *Grade II*: Isoechoic
- *Grade III*: Triple line/trilaminar
- *Grade IV*: Echogenic black region surrounding the midline echo

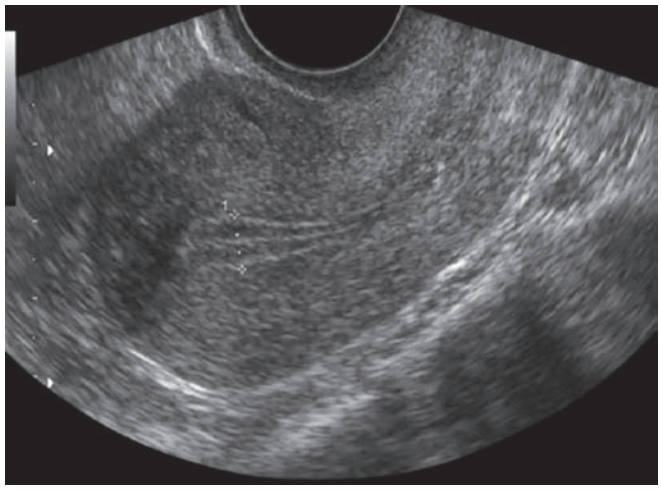
Further versions have been proposed subsequently which include a three grade system by Gonen and Casper in 1990.[7]

Type I: Entirely homogeneous, hyperechogenic pattern, without a central echogenic line

Type II: Intermediate iso-echogenic pattern, with the same reflectivity as the surrounding myometrium and a non-prominent or absent central echogenic line

Type III: Multilayered "triple-line" endometrium consisting of a prominent outer and central hyperechogenic line and inner hypo-echogenic or black region.

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.



Uterine Blood Flow: Doppler of the Uterine Arteries

A good blood supply towards the endometrium is usually considered to be an essential requirement for implantation and therefore, assessment of endometrial blood flow in IVF treatment has important role in IVF cycle.

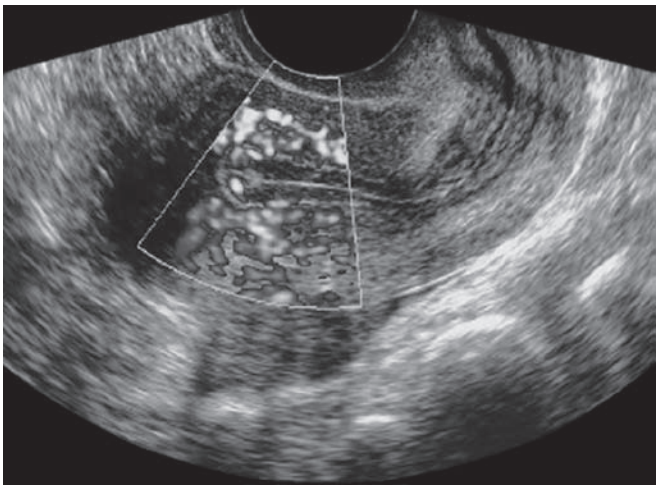
Uterine blood flow, as measured by colour Doppler, is suggested as a physiological parameter to assess receptivity. Colour Doppler signals are measured at the uterine arteries and their ascending branches located in the outer third of the myometrium[8] (Fleischer, 1991). The impedance of blood flow through the uterine arteries may be expressed as the pulsatility index (PI) and the resistance index (RI).

Changes in the endometrial vascularity appear present 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 per-iendometrial areas into the following four Zones:

1. Zone 1 – 2 mm thick area, surrounding the hyperechoic outer layer of the endometrium.
2. Zone 2 – The hyperechoic outer layer of the endometrium.
3. Zone 3 – The hypoechoic inner layer of the endometrium.
4. 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 Pre-transfer. If failure could be predicted, the embryos could be frozen until a more favourable cycle occurs. This could prevent embryo wastage and subsequent patient disappointment.

Sterzik et al[28] 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 was significantly lower than those who did not get pregnant.

Steer et al[29] 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[18] demonstrated a progressive decrease in the PI of the uterine arteries during the second half of the menstrual cycle in successful IVF pregnancies.[20]

In using color Doppler technique, inadequate vascular penetration of endometrial blood flow (not within zone 3) prior transfer has been associated with an unfavourable outcome. Vascular penetration towards the endometrial canal differs among patients. In patients with uterine artery PIs 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 with in 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.[28] In their study of 58 IVF patients a 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 the most likely to be associated with implantation. Smith et al[25] 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[22] 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* Non-miscarriage).

In the event of a 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.

Uterine Biophysical Profile

Certain sonographic qualities of the uterus are noted during the normal mid-cycle. These include:

- Endometrial thickness in greatest AP dimension of 7 mm or greater (full-thickness measurement).
- A layered ("5 line") appearance to the endometrium.
- Blood flow within zone 3 using color Doppler technique.
- Mymetrial contractions causing a wave like motion of the endometrium.
- Uterine artery blood flow, as measured by PI, less than 3.0.
- Homogeneous myometrial echogenicity.
- 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 :

- Endometrial thickness (full-thickness measured from the myometrial-endometrial junction to the endometrial-myometrial junction).
- Endometrial layering (i.e., a 5-line appearance).
- Myometrial contractions seen as endometrial motion.
- Myometrial echogenicity.
- Uterine artery Doppler flow evaluation.
- Endometrial blood flow.
- Gray-scale myometrial blood flow.

Each parameter is scored as follows:

- Endometrial thickness
  - < 7 mm = 0
  - 7-9 = 2
  - 10-14 mm = 3
  - > 14 mm = 1
- 2. Endometrial layering
  - No layering = 0
  - Hazy 5-line appearance = 1
  - Distinct 5-line appearance = 3
- 3. Myometrial contractions (seen as wave-like endometrial motion high-speed playback from videotape)
  - 3 contractions in 2 minutes (real-time) = 0
  - 3 contractions in 2 minutes (real-time) = 3
- 4. Myometrial echogenicity
  - Coarse/inhomogeneous echogenicity = 1
  - Relatively homogeneous echogenicity = 2
- 5. Uterine artery Doppler flow
  - PI-3.0 = 0
  - PI-2.99 = 0
  - PI-2.49 = 1
  - PI < 2 = 2
- 6. Endometrial blood flow within zone 3
  - Absent = 0
  - Present, but sparse = 2
  - Present multi-focally = 5
- 7. Myometrial blood flow internal to the arcuate vessels seen on gray-scale examination
  - Absent = 0
  - 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 dis-coordination). 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% 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 two spontaneous cycles (non-IVF, non-IUI), 2 IUI and 1 IVF). Scores of 17 to 19 (10 patients) have been associated with conception 80% of the time. Scores of 14 to 16 (10 patients) have a 60% 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 an 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 sub-groups based upon treatment protocol, age, cause etc.

The Experience at Malhotra Test Tube Baby Centre, Agra

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

- Scores of 17 to 19 (20 patients) conception rate of 84%.
- Score of 14 to 16 (20 patients) conception of 60%.
- 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 labs 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 investigated this, yet. We also do not believe that any group of findings will work perfectly 100% 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.

Experience at Malhotra Test Tube Baby Centre, Agra (2010)

RESULTS

	Spontaneous cycles n = 22		*COH + IUI n = 215*		*IVF-ICSI n = 62*		*Overall % of preg.*
	No.	*Preg.*	*No.*	*Preg.*	*No.*	*Preg.*	*Preg.*
Score 20	20	16 (80%)	35	30 (85%)	25	8 (83%)	82.6%
Score 17-19	93	41 (79%)	105	63 (60%)	30	3 (10%)	19%
Score 14-16	96	43 (44%)	61	33 (54%)	5	1 (20%)	39%
Score ≤ 13	13	1 (7.6%)	14	1 (7%)	2	0 (0%)	4.7%

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

With a perfect USSR Score of 20.97% 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.

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

ERA-ENDOMETRIAL RECEPTIVITY

It is a customised micro-array for genes involved in endometrial receptivity and it is predictive of endometrial dating. ERA Determines whether the sample is collected in Window of implantation and is receptive.

It compares the genetic profile of endometrium with that of LH + 7 in natural cycle and P + 5 in HRT (Diaz-Gimeno P et al. Fertil Steril 2011;95:50-60).

The result from the test will determine if a woman is receptive or not on the day and in the kind of cycle when the biopsy was performed. If she is receptive, it means that her window of implantation falls on the day of the cycle during which the biopsy was performed and, therefore, the blastocyst could implant on this day during the same kind of cycle. A non-receptive result could imply a displaced window of implantation. Therefore, a second biopsy would be needed to validate this displacement. For that, a specific day for the second biopsy will be suggested according to the first result obtained. This will allow the implantation in a subsequent cycle with a personalized embryo transfer

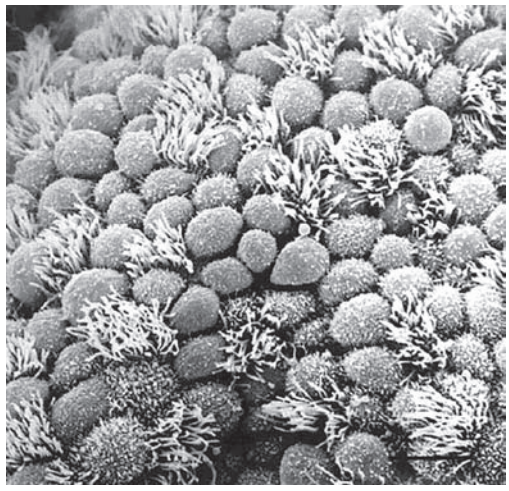
The ERA test has shown high sensitivity and specificity for detecting gene expression profiles associated with receptivity.

So what are Ideal Endometrial 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 and good embryos, embryo-endometrial interaction.

All these can be accurately predicted by biochemical assessments of markers, histological study of pinopodes. But 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 Trans-vaginal 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 or more receptive endometrium.

The consensus on how to do ideal endoemtrial blood flow study and endometrial receptivity scoring still tails [Appelebaun,[2] and Schild et al,[27] Kupesic,[30] Salle[31]].

CONCLUSION

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

REFERENCES

1. Applebaum M, Cadkin AV. Decidual flow – an early sign of pregnancy. Ultrasound Obstet Gynecol 1992;2:65.
2. Applebaum, M. The uterine biophysical profile (UBP). In: Allahabadia G, editor. Endosonography in obstetrics and gynaecology. Mumbai: Rotunda Medical Technologies (P) Ltd. 1997; 343-352.
3. Hertig AT, Rock J, Adams EC. A description of 34 human ova within the first 17 days of development. Am J Anat 1956 May;98(3):435-493.
4. Croxatto HB, Diaz S, Fuertealba BA, Croxatto HD, Carrillo D, Fabres C. Studies on the duration of egg transport in human oviduct. Fertil Steril 1972 Jul;23(7):447-458.
5. Buster JE, Bustilo M, Rodi IA, Cohen SW, Hamilton M, Simon JA, Thorneycroft IH, Marshall JR. Biologic and morphologic development of donated human ova recovered by nonsurgical uterine lavage. Am J Obstet Gynecol 1985 Sep;153(2):211-217.
6. Psychoyos A. Hormonal control of uterine receptivity for radiation. J Reprod Fertil 1976;25(Suppl):17-28.
7. Giudice LC. Potential biochemical markers of uterine receptivity. Hum Reprod 1999 Dec;14(Suppl 2):3-16.
8. Laird SM, Tuckerman EM, Dalton CF, Dunphy BC, Li TC, Zhang X. The production of leukemia inhibitory factor by human endometrium; presence in uterine flushings

and production by cells in culture. Hum Reprod 1997 Mar;12(3):569-574.
9. Sharkey AM, Dellow K, Blayney M, Macnamee M, Charnock-Jones S, Smith SK. Stage specific expression of cytokines and receptor messenger ribonucleic acids in human preimplantation embryos. Biol Reprod 1995 Oct;53(4):974-981.
10. Barano RI, Piazza A, Rumi LS, Polak de Fried E. Determination of IL-1 and IL-6 levels in human culture conditioned media. Am J Reprod Immunol 1997 Feb;37(2):191-194.
11. Lessey BA, YehL, Castelbaum AJ, Fritz MA, Ilesanmi AO, Korzeniowski P, Sun J, Chwalisz K. Endometrial progesterone receptors and markers of uterine receptivity in the window of implantation. Fertil Steril 1996 Mar;65(3):477-483.
12. Lessey BA, Castelbaum AJ, Sawin SW, Buck CA, Schinnar R, Bilker W, Strom BL. Aberrant integrin expression in the endometrium of women with endometriosis J Clin Endocrinol Metab 1994 Aug;79(2):643-649.
13. Lessey BA, Castel baum AJ, Sawin SW, Sun J. Integins as markers of uterine receptivity in women with primary unexplained infertility. Fertil Steril 1995 Mar;63(3):535-542.
14. Li TC, Dalton C, Hunjan K, Warren A, Bolton A. The correlation of placental problem 14 concentration in uterine flushing and endometeial morphology in the pre implantations period. Hum Reprod 1993 Nov;8(11):1923-1927.
15. De Loga JA, Krasnow JS, et al. Regional specialization of the cell membrane associated polymorpher mucin (MUC 1) in human uterine epithelia. Hum Reprod 1998;13:2902-2909.
16. Lindenberg S, Sundenberg K, et al. The milk degosaccharide Lacto-N-fucopentanose 1, inhibits attachment of mouse blastocya on endometrial monolayers. J Reprod Fertil 1998: 149-158.
17. Lindhard A, Ursula Bentin-Ley U, Ravn V, Islin H, Hviid T, Rex S, BangsbØll, SØrensen S. Biochemical evaluation of endometrial function at the time of implantation. Fertil Steril 2002 Aug;78(2):221-233.
18. Battaglia C, Larocca E, Lanzani A, Valentini M, Genazzani AR. Doppler ultrasound studies of the uterine arteries in spontaneous IVF stimulated ovarian cycles. Gynecol Endocrinol 1990 Dec;4(4):245-250.
19. de Ziegler D, Bessis R. Frydman R. Vascular resistance of uterine arteries : physiologic effects of estradiol and progesterone. Fertil Steril 1991 Apr;55(4):775-779.
20. Goswamy RK, Steptoe PC. Doppler ultrasound studies of the uterine artery in spontaneous cycles. Hum Reprod 1988;3: 721-726.
21. Goswamy RK, Williams G, Steptoe PC. Decreased uterine perfusion a cause of infertility. Hum Reprod 1988 Nov;3(8):955-959.
22. Kepic T, Applebaum M, Valle J. Preovulatory follicular size, endometrial appearance and estradiol levels in both conception and nonconclusion cycle a retrospective study. The 40th Annual Clinical Meeting of the Amerdin College of Obstetricians and Gynaecologists April 20 (abstract). 1992.
23. Rabinowitz R, Lauger N, Lewin A, Navot D, Bar I, Margalioth EJ, Schenker JJ. The value of ultrasonographic endometrial measurement in the prediction of pregnancy following *in vitro* fertilization. Fertil Steril 1986 Jun;45(6): 824-828.
24. Robertson WB. The endometrium. London, Boston: Butterworth; 1981.
25. Smith B, Porter R, Ahuja K, Craft I. Ultrasonic assessment of endometrial changes in stimulated cycles in an *in vitro* fertilization and embryo transfer program. J in Vitro Fertil Embryo Transf 1984 Dec;1(4):233-238.
26. Thichnan D, Arger P, Tureck R, Blasco L, Mintz M, Coleman B. Sonographic assessment of the endometrium in patients undergoing *in vitro* fertilization. J Ultrasound Med 1986 Apr;5(4):197-201.
27. Schild RL, Holthaus S, d'Alquen J, Fimmers R, Dorn C, van Der Ven H, Hansmann M. Quantilalve assessment of sub endometrial blood flow by 3-D ultrasound is on important predictive factor is an IVF programme. Hum Reprod 2000 Jan;15(1):89-94.
28. Sterzik K, Dallenback C, Schneider V, Sasse V, Dallinbach-Helweg G. In vitro fertilization : The degree of endometrial insufficiency varies with the type of ovarian stimulation. Fertil Steril 1988 Sep;50(3):457-462.
29. Steer CV, Campbell S, Pampiglione JS, Kingsland CR, Mason BA, Collins WP. Transvaginal color flow imaging of the uterine arteries during the ovarian and menstrual cycles. Hum Reprod 1990 Jun;5(4):391-395.
30. Kupesic S, Bekowal 1, Bjelos D, Kurjak A. Assesment of endometrial receptivity by TV Color Doppler and 3D PD ultrasonography in patients undergoing *in vitro* fertilization procedures. J ultrasound Med 2001 Feb;20(2):125-134.
31. Salle B, Bied-Damon V, Bencharb M, Desperes S, Gaucherand P, Rudigoz RC. Preliminary report of an ultrasonography and color Doppler uterine score to predict uterine receptivity in as *in-vitro* fertilization programme. Hum Reprod 1998 Jun;13(6):1669-1673.
32. Steer CV. Campbell S. Tan SL, Crayford T, Mills C, Mason BA, Collins WP. Transvaginal color Doppler: a new technique for us after *in vitro* fertilization to identify optimum uterine conditions before embryo transfer. Fertil Steril 1992 Feb;57(2): 372-375.