The Soil Test for Your Endometrium™: the Endometrial Function Test® (EFT®)

Harvey J. Kliman, MD, PhD

Yale University School of Medicine

A healthy pregnancy is like a successful garden. The successful garden starts with healthy plants and a nutrient rich soil. Likewise, the healthy pregnancy depends on a high quality embryo and a normal endometrium. Just as gardeners have always been able to test their soil to optimize plant growth, patients can now have their endometrium tested with the Endometrial Function Test® (EFT®). This patented test can optimize a patient's chances of having a successful pregnancy by using molecular markers to assess the endometrium's potential to support implantation and its ability to contribute to the nutrition of the developing embryo. To understand how the uterine soil performs its critical job we need to take a closer look at this unique tissue.

The Endometrium

The endometrium is made up of two components: the stroma and the glands (Figure 1). The endometrial stroma is the tissue that supports the glands and holds the endometrium together. It also contains the blood vessels that nourish the endometrial glands. In addition to its structural role, the stroma regulates the growth and function of the endometrial glands. The glands give the endometrium its special ability to mediate implantation. The glands, and the surface cells that are connected to the glands, make the initial contact with the embryo. If the glands are not functioning normally, the endometrium will not be receptive to the implanting blastocyst (the early embryo). Even if implantation were to occur, an abnormal endometrium is not able to support the nutritional needs of the early embryo, which may lead to early pregnancy loss. The endometrium is a unique tissue that, during a woman's reproductive years, grows, matures and then—if the woman is not pregnant—sloughs each month. Assessing these changes is the key to understanding the health of the endometrium.
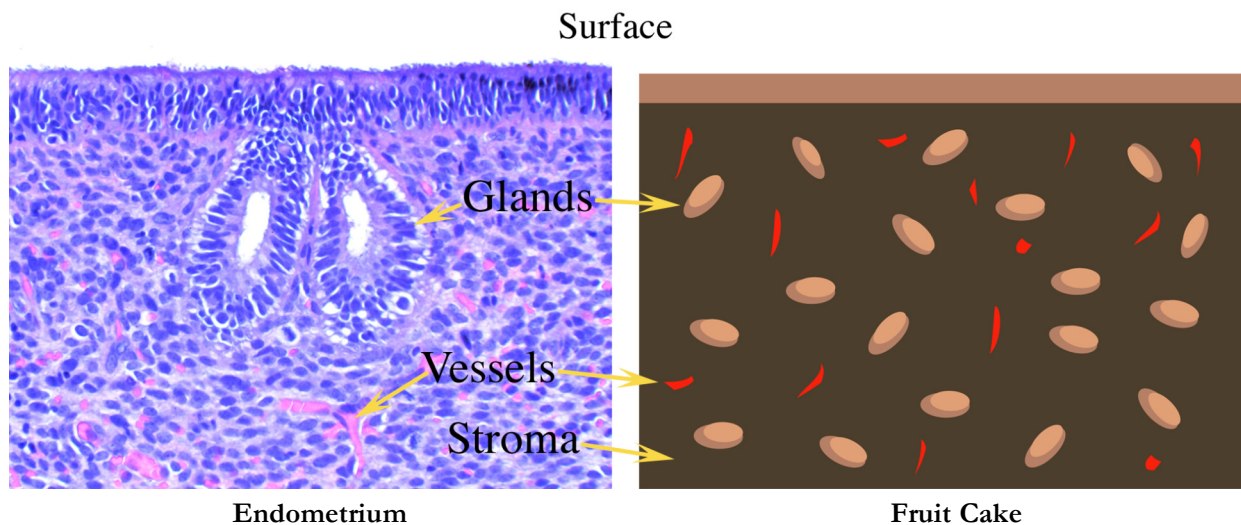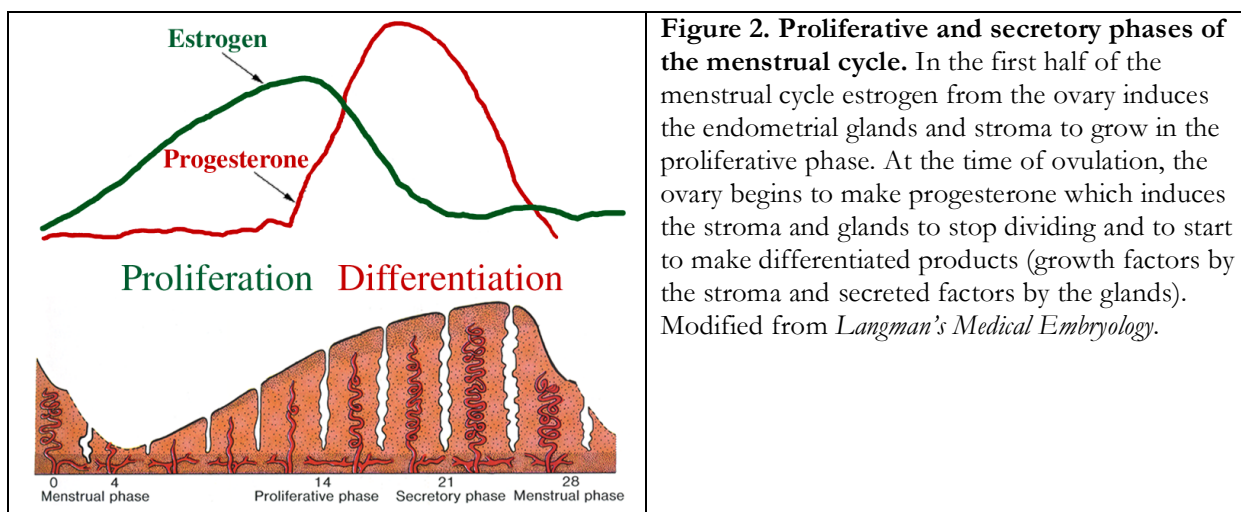

Surface

Glands

Vessels

Stroma

Endometrium   Fruit Cake

Figure 1. Components of the endometrium (left) compared to a fruit cake (right). The endometrium has two parts: the stroma and the glands. The stroma is the structural tissue that supports the embedded glands, just as the cake holds the fruit. The blood vessels, which supply nutrients to the endometrium, are also found within the stroma. In addition to their structural role, the stromal cells produce growth factors and hormones that regulate the glands. The glands, and the surface cells which are connected to the glands, make the initial contact with the implanting blastocyst. Note the secretory vacuoles (cleared areas) within the gland cells, which is typical of cycle day 16. Defects in glandular function lead to an unreceptive endometrium which can not support implantation.

During each menstrual cycle, a women's endometrium goes through dramatic changes necessary to prepare for implantation. The first half of each cycle is a time of cell growth called the proliferative phase (Figure 2). In a natural cycle this phase is controlled by the estrogen produced in the ovary between cycle days 1 and 14. Estrogen acts on both the stroma and the glands to induce these cells to divide, causing the endometrium to thicken—something that can be seen by vaginal ultrasound. In the absence of estrogen the endometrium remains dormant and does not make the components that are necessary for implantation. Around the time of ovulation—which occurs on cycle day 14 in an idealized 28 day cycle—the ovary begins to make progesterone. Progesterone first causes both the stroma and glands to stop growing (proliferating) and then it induces these tissues to change (differentiate) into the mature forms necessary to support implantation. The first visible microscopic change in the glands at this time is the appearance of secretory vacuoles (see glands in Figure 1), which is why the later part of the menstrual cycle is known as the secretory phase (sometimes also called the luteal phase). Studies have shown that progesterone induces the stroma to make special growth factors that communicate with the glands. This communication is crucial for the proper development of the endometrium.
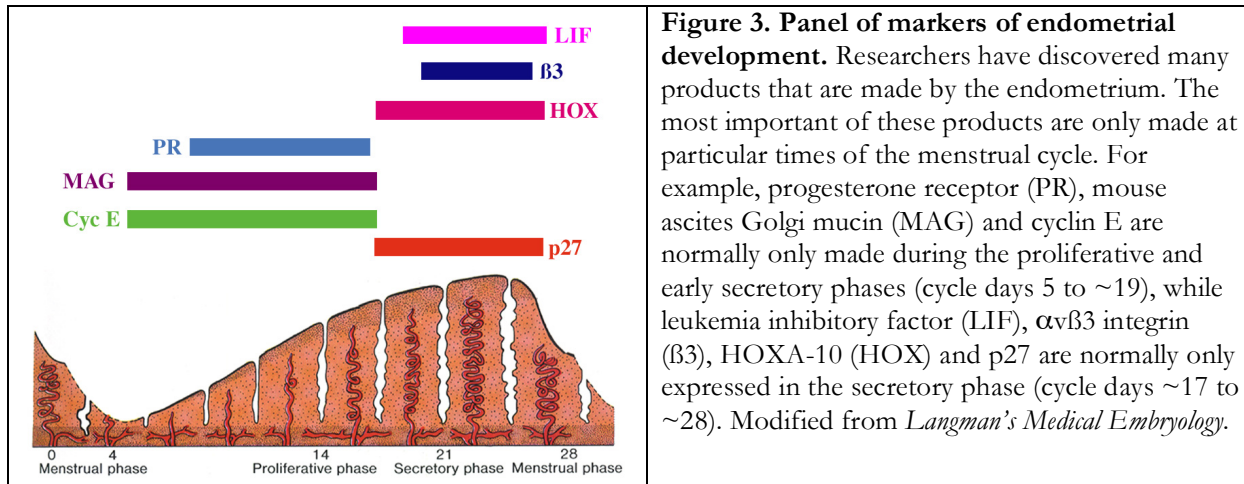

Figure 2. Proliferative and secretory phases of the menstrual cycle. In the first half of the menstrual cycle estrogen from the ovary induces the endometrial glands and stroma to grow in the proliferative phase. At the time of ovulation, the ovary begins to make progesterone which induces the stroma and glands to stop dividing and to start to make differentiated products (growth factors by the stroma and secreted factors by the glands). Modified from *Langman's Medical Embryology.*

Assessing the endometrium

Currently the only way to assess all the components of the endometrium is to examine a small sample under the microscope. Traditionally the pathologists who do this examination routinely process the endometrial biopsy that is collected by the gynecologist or reproductive endocrinologist by first fixing the tissue and then staining it with two dyes: hematoxylin and eosin (H&E, see Figure 1 for an example). Unfortunately H&E staining only shows the overall structure of the endometrium, it does not detect the crucial growth factors that control endometrial development.

Over the last 10-15 years researchers have discovered many of the crucial growth factors made by the endometrium that play a role in the implantation process (Figure 3). Utilizing insights gained from examination of the mediators of blastocyst implantation, researchers have elucidated a series of markers that can be used to assess the functional state of an endometrial sample. Each marker has an established period when it is normally expressed.

Figure 3. Panel of markers of endometrial development. Researchers have discovered many products that are made by the endometrium. The most important of these products are only made at particular times of the menstrual cycle. For example, progesterone receptor (PR), mouse ascites Golgi mucin (MAG) and cyclin E are normally only made during the proliferative and early secretory phases (cycle days 5 to ~19), while leukemia inhibitory factor (LIF), αvß3 integrin (ß3), HOXA-10 (HOX) and p27 are normally only expressed in the secretory phase (cycle days ~17 to ~28). Modified from *Langman's Medical Embryology.*

Although markers that assess both the stroma and the glands have been explored, the most important markers look exclusively at the glands. The reason for this is that the glands have been shown to be the first cells that interact with the implanting blastocyst and therefore the state of the glands most accurately reflects the receptivity of the endometrium. Of these glandular markers, currently only αvß3 integrin (ß3) is commercially available for endometrial assessment. However, the utility of this marker has been recently questioned. Progesterone receptor (PR) has been available for some time, but its expression alone has not been shown to reliably predict endometrial receptivity. Leukemia inhibitory factor (LIF) has been shown to be critical for mouse implantation and has also been shown to vary throughout the human menstrual cycle, but it has not been shown to be predictive of endometrial receptivity. HOXA 10 also varies throughout the menstrual cycle and appears to be altered in the unreceptive endometrium, but currently this marker cannot be assessed with standard techniques. The availability of antibodies to the HOXA 10 product at some point in the future may change this. Mouse ascites Golgi mucin (MAG), a specific carbohydrate epitope on the MUC1 mucin found in many tissues, has been shown to predict endometrial receptivity. Its use, however, is limited to patients with blood types A or AB. Selectins, which have been shown to mediate both white blood cell attachment to blood vessel walls and the earliest phases of implantation, have not been tested clinically. The limitations of all these markers has stimulated continued research to discover reliable markers that can be used in all patients. The most promising markers to date appear to be the cyclins, which are the basis of the Endometrial Function Test® (EFT®).

The cyclins regulate cell growth and come in pairs, one that promotes growth and one that inhibits growth. We have found that cyclin E, which enhances endometrial growth, and p27, which inhibits it, are the most useful cyclins to examine the endometrium. Fertile women express cyclin E in the first half of the cycle when the endometrium grows in thickness and p27 in the second half of the cycle when the endometrium matures (Figure 4). Based on this observation we have concluded that estrogen stimulates the appearance of cyclin E, while progesterone causes cyclin E to disappear and p27 to appear. The patterns of cyclin E and p27 expression appear to be very different in women with unexplained infertility (Figure 5). The persistent expression of cyclin E into the secretory (luteal) phase of the endometrial cycle suggests that the glands arrested (stopped their development) sometime earlier, possibly because of a premature expression of p27. The specific pattern of staining seen in many abnormal endometrial biopsies suggests that this *glandular developmental arrest* (GDA) occurs most frequently around cycle day 18, the cycle day that both cyclin E and p27 are present at the same time.
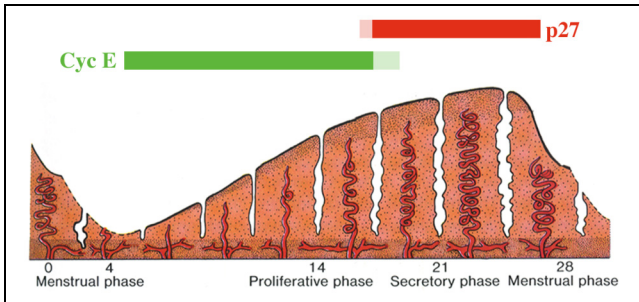
Figure 4. Cyclin E and p27 expression in fertile women. Cyclin E first appears at around cycle day 5 and continues to be expressed up until cycle day 19. After day 19, cyclin E normally is absent. p27, on the other hand, is absent until approximately cycle day 17, where it is seen for the remainder of the cycle. Modified from *Langman's Medical Embryology.*
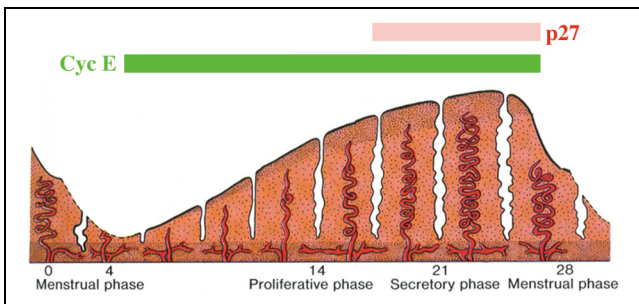

Figure 5. Cyclin E and p27 expression in women with unexplained infertility. The most striking difference between the cyclin expression of fertile women and infertile women is the persistence of cyclin E and decreased presence of p27 into the secretory phase. This finding represents a developmental arrest of the glands in the endometria of these women. Modified from *Langman's Medical Embryology.*

Why do we see glandular developmental arrest (GDA) so commonly in cases of unexplained infertility? The stroma, which is much more than a medium to hold the glands, communicates with the glands to control their growth and development. When estrogen and progesterone enter the endometrium, they first interact with the stroma. It is known that many of the factors necessary for glandular growth and development come from the stroma. When the communication between the stroma and glands is working, it is like a surfer successfully catching a wave. If there is a breakdown in the normal stromal to glandular communication the glands miss the stromal wave and are left behind like a stranded surfer (Figure 6).
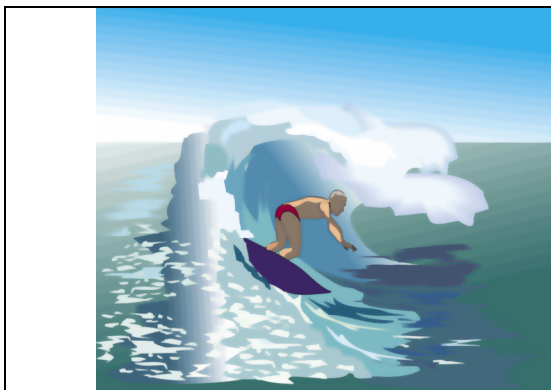

Figure 6. The surfer and the endometrium. A normal endometrium is like a surfer and the wave he has caught—with the wave being the stroma and the surfer being the glands. Just as a surfer will miss the wave if it goes by too quickly, the endometrial glands can be left behind if the stroma moves too quickly. This can happen when there is too much progesterone or the stroma is too sensitive to the amount of progesterone present. Giving progesterone in a more gradual fashion can help the glands "catch" the developing stroma.

Treatment options to improve endometrial receptivity

The two most rapidly evolving treatment strategies for implantation defects are optimization of steroid hormone protocols and elimination of extrauterine factors that interfere with endometrial development. Every patient is unique, and the treatments necessary to heal her endometrium must be individually crafted.

The hormones estrogen and progesterone always have been acknowledged as critical for implantation. Women undergoing infertility treatments that lower progesterone production, such as GnRH agonist (Lupron™) and egg retrieval for IVF, require progesterone supplementation to achieve pregnancies. More controversial has been the issue of whether subtle variations in the doses and/or duration and/or routes of administration influence implantation.

Studies comparing the effects of hormone preparations and routes of administration on markers of endometrial receptivity provide a sensitive indicator of the effects of hormones on the implantation process. When examined under the microscope with routine H&E processing, the structure of the endometrium appears more normal after vaginal versus intramuscular routes of progesterone administration. However, relatively few useful endometrial abnormalities can be identified with routine H&E processed tissue. On the other hand, the EFT® is a far more sensitive tool to diagnose endometrial defects because it examines endometrial functions that are specifically mediated by both estrogen and progesterone. Using the EFT® as a guide, therefore, we have been able to alter the doses and durations of the steroid hormones and hence individualize treatment for each patient. While systematic study of these treatment strategies still are under way, we have had promising early successes for some very challenging patients.

Other factors may disrupt implantation by indirectly altering the uterine lining. One example of this is hydrosalpinx. When the ends of the Fallopian tubes become obstructed, e.g. from previous infection or endometriosis, normal secretions from cells lining the Fallopian tube accumulate, become stagnant, then leak back into the uterus, where they interfere with implantation. Removing or draining a hydrosalpinx may be a critical first step in promoting normal implantation in these patients. Like hydrosalpinx, there is also evidence that endometriosis disrupts endometrial development, and hence implantation. Medical and/or surgical treatment of endometriosis has been shown to improve pregnancy rates for some patients. There is evidence that some women may have implantation difficulties because of either a too low or too high Body Mass Index (BMI), possibly related to how these women respond to food intake and their own insulin production. Finally, there is evidence that stress may be harmful to implantation and subsequent pregnancy. Hopefully, as tests of endometrial receptivity, such as the EFT, become more available, the decision of who should undergo removal of their damaged Fallopian tubes, treatment for endometriosis, nutritional intervention, and/or stress reduction programs will become a more precise and a more individualized process.

Conclusions

For patients who have not been able to achieve a successful pregnancy, either on their own or with some form of assisted reproductive technology, the answer may lie in their uterine soil, the endometrium. Based on two awarded patents, the Endometrial Function Test® (EFT®) may be the most efficient way to assess endometrial receptivity and guide therapies prior to patients undergoing expensive assisted reproductive technology procedures.

Additional Information

For additional information on the Endometrial Function Test**®** (EFT®), please go to: *http://info.med.yale.edu/obgyn/kliman/* and click on *Infertility Research* and then the link to the *Endometrial Function Test***®** *(EFT®).* There you will find a pdf of this article and more information on the EFT. You can also call Dr. Kliman's laboratory at Yale at 203-785-7642.

References

1. RL Dubowy, HS Taylor, EA Nannenberg, H Zhang, HJ Kliman. Cyclin E and p27 are potential markers of endometrial receptivity. 2000 Annual Meeting of the Society for Gynecologic Investigation, Chicago, IL.
2. Kliman H, Dubowy R, Feinberg R. Improved accuracy of endometrial assessment using cyclin E and p27. Human Fertility and Reproduction: the oocyte, the embryo and the uterus. New York University, NY, NY, 2000.
3. Dubowy RL, Feinberg RF, Kliman HJ. Endometrial biopsy and infertility evaluation: new insights from cyclins. American Society for Reproductive Medicine, San Diego, CA, 2000.
4. Kliman HJ, Spandorfer SD, Barmat LI, Rosenwaks Z, Catalanotti JS, McSweet JC. MAG mucin expression abnormalities in natural cycle biopsies predict subsequent IVF failure. 2001 Annual Meeting of the Society for Gynecologic Investigation, Toronto, ON.
5. Kliman HJ, McSweet JC, Grunert GM, Cardone VRS. Efficacy of the Endometrial Function Test (EFT) in Assisted Reproductive Technology (ART) Practice. 2002 Annual Meeting of the American College of Obstetricians and Gynecologists, Los Angeles, CA.
6. Kliman HJ, McSweet JC, Grunert GM, Cardone VRS, Cadesky K, Keefe DL. The Endometrial Function Test (EFT)™ Directs Care and Predicts ART Outcome. American Society for Reproductive Medicine, Oral #44, Seattle, Washington, 2002.
7. Kliman HJ, Adel T, Isaac BR, Khosla N, McSweet JC, Franco A, Taylor HS, Santoro NF. Menstrual, Hormonal and Endometrial Evaluation of a Woman Traversing the Menopause: Frequent and Silent Anovulation and Endometrial Dysfunction. 2003 Annual Meeting of the Society for Gynecologic Investigation, Washington, DC.
8. Dubowy RL, Feinberg RF, Keefe DL, Doncel GF, Williams SC, McSweet JC, Kliman HJ. (2003) Improved endometrial assessment using cyclin E and p27. Fertil Steril, 80:146-56.
9. Sarno JL, Kliman HJ, Taylor HS. PBX2 and MEIS 1 are HOXA10 co-activators in human endometrium. 2004 Annual Meeting of the Society for Gynecologic Investigation, Houston, TX.
10. Lorch AC, Franco A, McSweet JC, Keefe DL, Kliman HJ. Telomerase expression in fertile patients compared to infertile patients with thin endometria. Oral presentation at the 60th Annual Meeting of the American Society for Reproductive Medicine (ASRM), October 16-20, 2004, Philadelphia, PA.
11. Stephenson M, Houlihan E, Karrison T, Franco A, McSweet JC, Kliman HJ. Successful treatment of endometrial-associated recurrent pregnancy loss (RPL): A blinded, controlled study. Oral presentation at the 60th Annual Meeting of the American Society for Reproductive Medicine (ASRM), October 16-20, 2004, Philadelphia, PA.
12. Boivin J, Schmidt L. (2005) Infertility-related stress in men and women predicts treatment outcome 1 year later. Fertil Steril, 83:1745-52.