

The Development and Utilization of the Endometrial Function Test™ (EFT®)

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There are many hurdles to overcome to achieve a normal pregnancy. Possibly the most difficult step of all is the attachment (implantation) of the embryo to the uterine lining—also called the endometrium. Abnormalities in the process of implantation are believed to be the basis of many cases of unexplained infertility in women. The question that researchers are trying to answer is: Can we help women for whom implantation has not been successful? To do so, we need to be able to distinguish between a functionally normal and abnormal endometrium—and to do that we need to understand the functions of each of the parts of the endometrium.

The Endometrium

The endometrium is made up of two components: the stroma and the glands. If you imagine a fruit cake, the stroma is the cake and the glands are the fruit embedded in the cake (Figure 1). Like the cake, 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 leads to early pregnancy loss. The endometrium is a unique tissue that grows, matures and then—if the woman is not pregnant—sloughs each month during her reproductive years. Assessing these changes is the key to understanding the health of the endometrium.

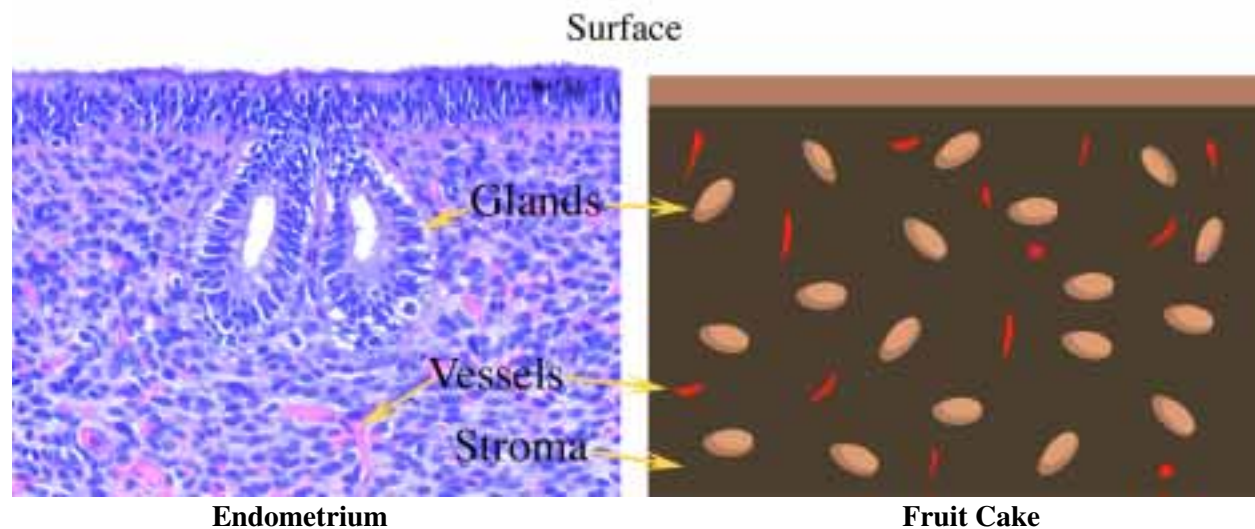
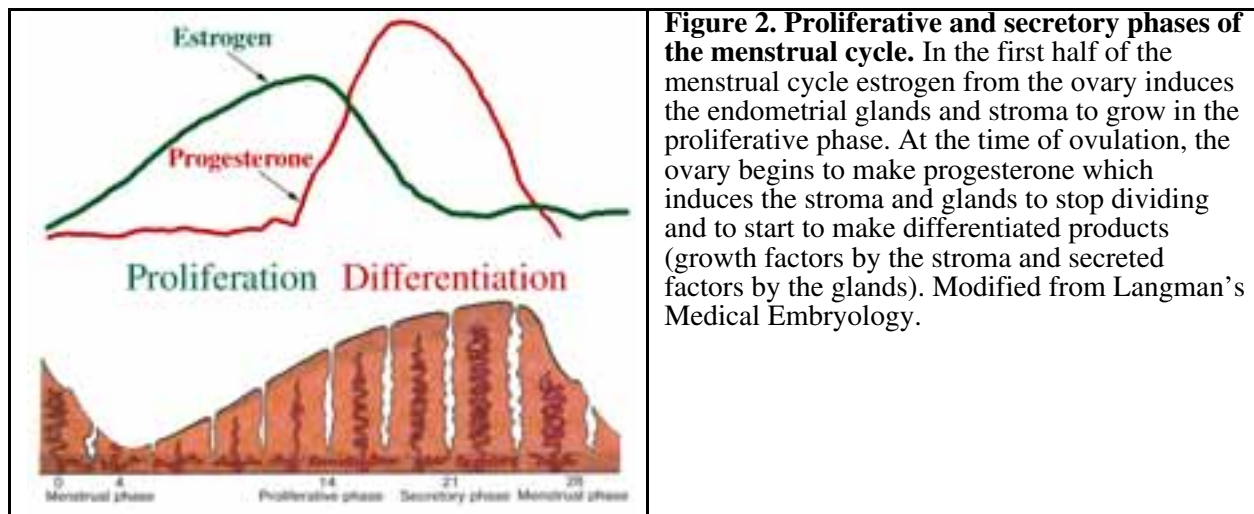


Figure 1. Components of the endometrium (left) compared to a fruit cake (right). The endometrium has two parts: the stroma and the glands. The stroma is the structural tissue that supports the embedded glands, just as the cake holds the fruit. The blood vessels, which supply nutrients to the endometrium, 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.

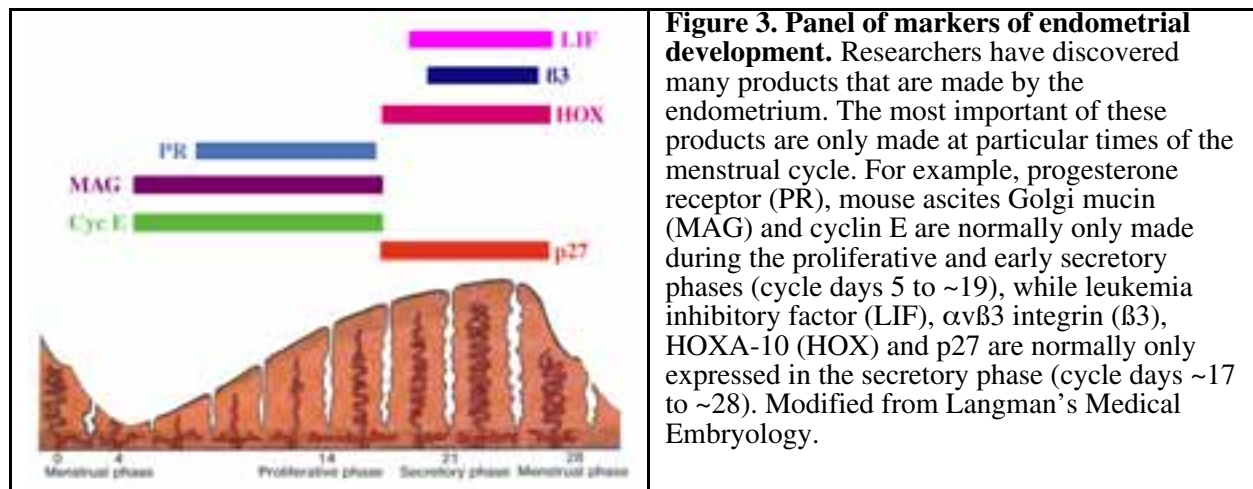
During each menstrual cycle, a women's endometrium goes through dramatic changes necessary to prepare for implantation. The first half of each cycle is a time of cell growth called the proliferative phase (Figure 2). In a natural cycle this phase is controlled by the estrogen produced in the ovary between 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 their 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 performed in research laboratories have indicated that in addition to acting directly on the glands, progesterone acts indirectly through the stroma. In other words, progesterone induces the stroma to make special growth factors that communicate with the glands. Even with sufficient progesterone, the glands also need these stromal factors to fully mature (Lessey, 2000).



Assessing the endometrium

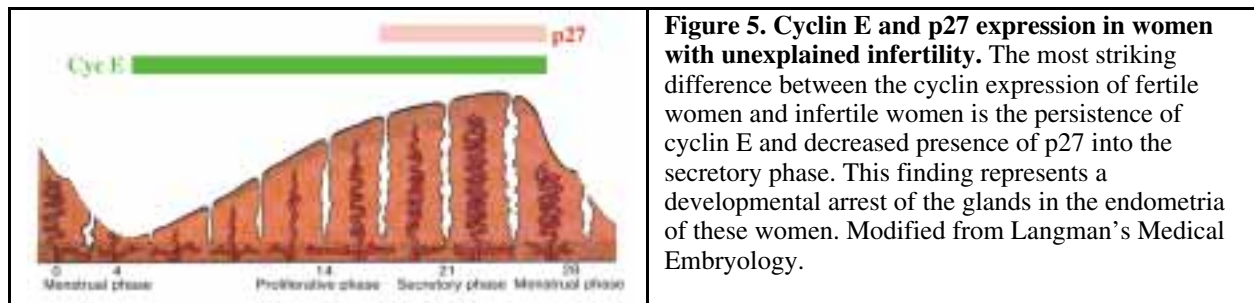
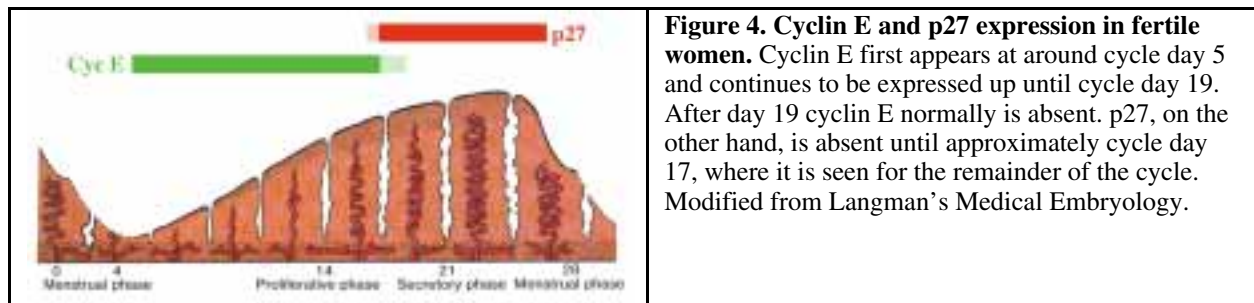
Currently the only way to assess all the components of the endometrium is to examine a small sample under the microscope. Traditionally the pathologists who do this examination routinely process the endometrial biopsy that is collected by the gynecologist or reproductive endocrinologist by first fixing the tissue and then staining the resultant sections with two dyes: hematoxylin and eosin (H&E). This tie-dying-like procedure leaves the tissue blue and red (see Figure 1 for an example). For over 50 years this has been the only practical way to assess endometrial function (Noyes et al., 1950). Unfortunately this crude staining procedure is unable to observe the affects of the stromal and glandular communications that are critical for normal endometrial development.

Over the last 10-15 years researchers have discovered many 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. Combining two or more of these markers together establishes an Endometrial Function Test™ (EFT®)(Dubow et al., 2003).



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 $\alpha v\beta 3$ integrin ($\beta 3$) is commercially available for endometrial assessment. However, the utility of this marker has been recently questioned (Thomas et al., 2003). Progesterone receptor (PR) has been available for some time (Lessey et al., 1996), but its expression 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 (Arici et al., 1995), but it has not been shown to be predictive of endometrial receptivity. HOXA 10 also varies throughout the menstrual cycle (Taylor et al., 1998) and appears to be altered in the unreceptive endometrium (Taylor et al., 1999), 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 endodermal tissues (Kliman et al., 1995), has been shown to predict endometrial receptivity (Kliman et al., 1999; Kliman et al., 2001). Its use, however, is limited to patients who are blood types A or AB. Selectins, which have been shown to mediate both white blood cell attachment to blood vessel walls (Jung et al., 1998) and the earliest phases of implantation (Genbacev et al., 2003; Lessey and Young, 1997), 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 (Dubow et al., 2003; Kliman et al., 2002).

The cyclins regulate cell growth and come in pairs like the gas and brake pedals in a car. We have found that cyclin E (the gas pedal cyclin) and p27 (the brake cyclin) are the most useful cyclins to examine in the endometrium (Dubowy et al., 2003). Fertile women express cyclin E in the first half of the cycle when the endometrium develops 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 (Dubowy et al., 2003). The patterns of cyclin E and p27 expression appear to be very different in women with unexplained infertility (Figure 5) (Dubowy et al., 2000a; Dubowy et al., 2000b; Kliman et al., 2000). 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 brake). The specific pattern of staining seen in many endometrial biopsies suggests that this glandular developmental arrest (GDA) occurs most frequently around cycle day 18 (Kliman et al., 1997).



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, controls how the glands grow and develop. 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, but everything must work together smoothly, like a surfer catching a wave (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 will be different.

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 (Devroey and Pados, 1998). However, relatively few useful endometrial abnormalities can be identified with routine H&E processed tissue (Network, 2002). On the other hand, the EFT, mentioned above, is a far more sensitive tool to diagnose endometrial defects because it examines endometrial functions that are specifically mediated by both estrogen and progesterone (Dubowy et al., 2003). 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 (Kliman et al., 2002).

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 (Strandell and Lindhard, 2002). 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. 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 or treatment for endometriosis will become a more precise and a more individualized process.

Conclusions

Infertility is an emotionally devastating medical problem for couples who wish to have children. Infertility treatment demands significant time and financial resources from couples facing this condition. Currently, we can evaluate male fertility by testing semen function with direct microscopic examination and sperm function with *in vitro* fertilization testing. Until recently, no such tests have been available for endometrial receptivity. Based on awarded and pending 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.

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