Clinical approach to recurrent implantation failure: evidence-based evaluation of the endometrium

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The endometrium is a dynamic, repetitively cycling tissue that mediates the implantation of the blastocyst. Evaluation of this complex tissue necessitates sophisticated methods that can assess its functional potential. Beginning in the 1950s with simple histological endometrial "dating," these methods have crossed into the molecular era with the use of arrays aimed at dating, functional tests that assess for proliferation and differentiation, and tests that screen for inflammatory markers. In addition to these specialized tests, histologic evaluation for pathologic conditions—such as growth disorders (i.e. polyps and hyperplasia), inflammatory lesions, and retained products of conception—are critical for a complete assessment of the patient with recurrent implantation failure. Whatever the means of testing, the goal is to reveal actionable findings that can assist in offering the best options to patients who have failed multiple transfers with high quality embryos. (Fertil Steril® 2019;111:618–28. ©2019 by American Society for Reproductive Medicine.)
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The human endometrium is a unique tissue in our species. No other tissue proliferates, differentiates, sloughs—over and over again, cycle after menstrual cycle for up to 45 years. This complex transformation is the direct result of the endometrium's multifunctional task: to be contemporaneously receptive to the developing embryo in cycles with successful fertilization; to, on the one hand, allow for the attachment of the blastocyst and the ensuing trophoblast invasion; and, on the other hand, prevent overly aggressive incursion by the invasive trophoblasts, which, if not checked, could penetrate and rupture the uterus; and finally to shed when there is no implantation—only to start all over again in anticipation of the next potential pregnancy.

No other tissue in our species has the task of radically changing its biology, endocrinology, structure, and function in this way, let alone repeatedly. This is why it is no simple task to assess the function of the endometrium, to understand or diagnose the reasons for its abnormalities, and devise treatments that can repair an endometrium that does not appear to be functioning correctly.

If the endometrium, the receptive surface lining the inner layer of the uterus, were simply a layer of adhesive glue, there would not be much to assess or fix. However, such a nonspecific adhesive layer would be continuously permissive, with no selectivity. Any embryo entering the uterine cavity would implant and result in a pregnancy, whatever the quality of the embryo, or the state of the woman whose uterus has been entered (1). It is the sheer complexity of the endometrium that gives it its precise and selective potential, which in turn allows for a developmentally competent embryo to gain a foothold at a time when the gravida is most able to support a successful pregnancy.

IMPLANTATION WINDOW

The interval during the menstrual cycle when a developmentally competent embryo is permitted to attach to, and ultimately invade into, the endometrium has been termed the "implantation window." The implantation window was first recognized by reproductive endocrinologists when they assessed the timing of an embryo transfer. Navot et al. (2) elegantly documented the fact that 42 to 48 hour old embryos only implanted when transferred on cycle days (CDs) 17, 18 or 19 (normalized to CD

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14 being ovulation day; Fig. 1). Earlier or later transfers did not result in any pregnancies. Further studies (3, 4) refined this observation demonstrating that, with assisted reproductive technology, the window of implantation spanned CDs 19–23 (Fig. 1). These findings correlated well with direct observations of 4.5–7 day old human embryos implanting in naturally cycling fertile women between CDs 18.5 to 21 (5). In addition, an in vitro co-culture model involving purified human cytotrophoblasts and endometrial explants revealed that trophoblasts non-selectively adhere to exposed stroma irrespective of CD. However, trophoblasts only adhered to the endometrial surface epithelium from samples collected on CD 19 (6), indicating that the surface epithelium is the most critical component of endometrial selectivity.

It is clear that implantation is not a single event, but, more accurately, a cascade of interactions between the embryo's trophectoderm, endometrial surface, and glandular epithelium (7, 8). Mechanistically, implantation appears to be similar to the well-described interactions between leukocytes and endothelium. Leukocytes initially interact with the endothelial surface with the rolling reaction, followed by tight binding, and culminating in cytokinesis through the endothelial layer (9). Likewise, upon arriving in the endometrial cavity, the embryo first weakly opposes the endometrial surface epithelium through interactions with long mucin molecules (apposition phase) (10). Next, the blastocyst tightly adheres to the endometrial surface via the interaction of trophoblast derived fetal fibronectin (11) with endometrial adhesion molecules, such as the α_v/β_3 integrin (ß3) (adhesion phase) (12, 13). This process culminates with trophoblast invasion through the epithelium and into the endometrial stroma (5, 14). This implies that both the embryo and endometrium must sequentially and synchronously

FIGURE 1

Cycle day by dating	LH Surge	LH Dating	Ovulation	Embryo Development in days and hours	Day of P exposure	P dating	EFT Biopsy	ERA Biopsy	ReceptivaDx Biopsy	Embryo Transfer
Days 1-12										
Day 13	LH surge	LH+0								
Day 14		LH+1	Ovulation	Day 0; fertilization	Day 1 (first full day)	P+0				
Day 15		**LH+2**		**Day 1 (24 hours old); fertilization check**	**Day 2**	**P+1**	**d15 biopsy**			
Day 16		LH+3		Day 2 (48 hours)	Day 3	P+2				
Day 17		**LH+4**		**Day 3 (72 hours)**	**Day 4**	**P+3**				**day 3 embryo transfer**
Day 18		LH+5		Day 4 (96 hours)	Day 5	P+4				
Day 19		**LH+6**		**Day 5 (120 hours)**	**Day 6**	**P+5**		**P+5**	**LH+6–LH+10 or P+5–P+10**	**day 5 blastocyst transfer**
Day 20		LH+7		Day 6 (144 hours)	Day 7	P+6		**LH+7**		
Day 21		LH+8		Day 7	Day 8	P+7				
Day 22		LH+9		Day 8	Day 9	P+8				
Day 23		LH+10		Day 9	Day 10	P+9				
Day 24		**LH+11**		**Day 10**	**Day 11**	**P+10**	**d24 biopsy**			
Day 25		LH+12		Day 11	Day 12	P+11				
Day 26		LH+13		Day 12	Day 13	P+12				
Day 27		LH+14		Day 13	Day 14	P+13				
Day 28: menses		LH+15		Day 14	Day 15	P+14				

Endometrium embryo progesterone testing timing. The cycle day by dating column represents an idealized 28-day menstrual cycle. The key milestones for endometrial dating are shown in red (LH surge, day of ovulation, and first full day of progesterone). The light orange shaded columns represent the three major endometrial assessment tests, with the blue shaded cells being the suggested endometrial sampling days based on literature supplied by the testers (for the EFT: https://medicine.yale.edu/obgyn/kliman/infertility/eft/providers.aspx#page3; for ERA: https://www.igenomix.com/hubfs/493037/.com/era_specialist_protocol.pdf?hsLang=es-es; for the ReceptivaDx: https://www.receptivadx.com/biopsy-information). The light green shaded column matches the day 3 and 5 embryo transfers with the cycle day by dating column. EFT = endometrial function test; ERA = endometrial receptivity analysis; LH = luteinizing hormone; P = progesterone.

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acquire functional components or implantation will not be achieved. The complexity of the endometrium's dynamically transformative machinations is manifested in its complex structure.

STRUCTURE DETERMINES FUNCTION AND ENDOMETRIAL DATING

While the lining of the tracheal bronchial tree is made up of a single epithelial layer of ciliated columnar cells and mucus secreting goblet cells, the endometrium is a mixture of glandular epithelial cells embedded in a mesenchymal stroma. This bipartite architecture resembles "fruitcake" (Fig. 2), with the glands represented by "fruit" embedded within the "doughy" stroma. The endometrial glands begin as invaginations from the epithelial cell covered surface into a vascular stroma. This mesenchymal stroma is both hormonally responsive and secretory with a constantly changing population of bone marrow derived immune and stem cells.

These two cellular compartments, epithelial gland and mixed stroma, change on a daily basis in an endocrine paracrine dance. The final target of the hypothalamic pituitary ovarian axis—the endometrium—receives exogenous endocrine signals that impact the stromal cells, which in turn generate paracrine signals inducing the embedded epithelial cells to grow, differentiate, secrete, and finally become quiescent (15).

While the glands finish their visible function on CD 19, the stroma begins to transform the endometrium from receptive to an invasion resistant decidualized barrier (16–18). Remarkably, if there is no embryonic nidation, the stroma must self-destruct—a process mediated by autolytic enzymes and vasoactive substances that result in controlled endometrial sloughing (18). With the synchronized resumption of ovarian estradiol release, the cycle resumes with post sloughing endometrial proliferation. It is this exquisitely timed and continuously changing interplay between stroma and glands that allowed Noyes, Hertig and Rock to "date" the endometrium (Fig. 3) (19).

The initial schema for endometrial dating published as the first article in *Fertility and Sterility* provided a tool with the potential to differentiate between a normal and abnormal endometrium. For many years endometrial histologic dating served as a key part of the infertility evaluation (21). However, it became apparent that histologic dating alone was not sufficient to determine the etiology of implantation failure (22, 23). In 2004, the Cooperative Reproductive Medicine Network noted that out of phase biopsies could not discriminate between fertile and infertile women. In fact, fertile women had a statistically non-significant higher proportion of out of phase biopsies (24). These results highlight that histological dating alone lacks the sensitivity to identify a definable defect in endometrial development and, therefore, in the implantation process.

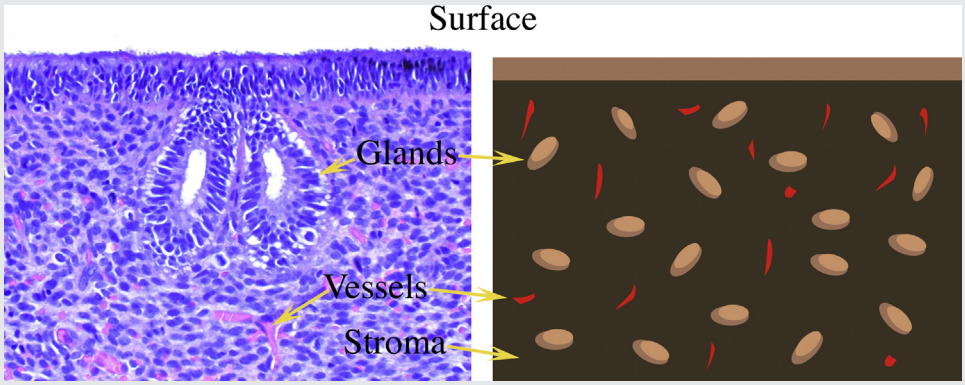
HISTOPATHOLOGY AND IMPLANTATION

It is simplistic to assume that the only problem a patient faces in terms of implantation failure is one of dating. This view is proven false by the varied pathologies found in women's endometrial biopsies (25). Figure 4 illustrates but a few of the more common pathologic findings seen in patients seeking infertility treatment. These can be divided into the following general categories: growth disorders, inflammatory lesions, autoimmune lesions, and retained products of conception.

Growth Disorders

We assume that exposure to either endogenous or exogenous estrogen (E) and progesterone (P) will lead to

FIGURE 2



Fruitcake model of the endometrium. The endometrium consists of two distinct cell types: a mesenchymal stroma (the brown cake) and surface (icing) derived epithelial glands (the pieces of fruit). Exogenous hormones arrive via afferent vessels, which permeate the stroma (candied cherries of a fruitcake). Hormones modulate the stromal cells, which then produce paracrines, growth factors, and cytokines that control glandular proliferation and differentiation. The afferent vessels are also the point of entry of exogenous immune and non-immune cells that become incorporated into the endometrial stroma in a cycle dependent manner.

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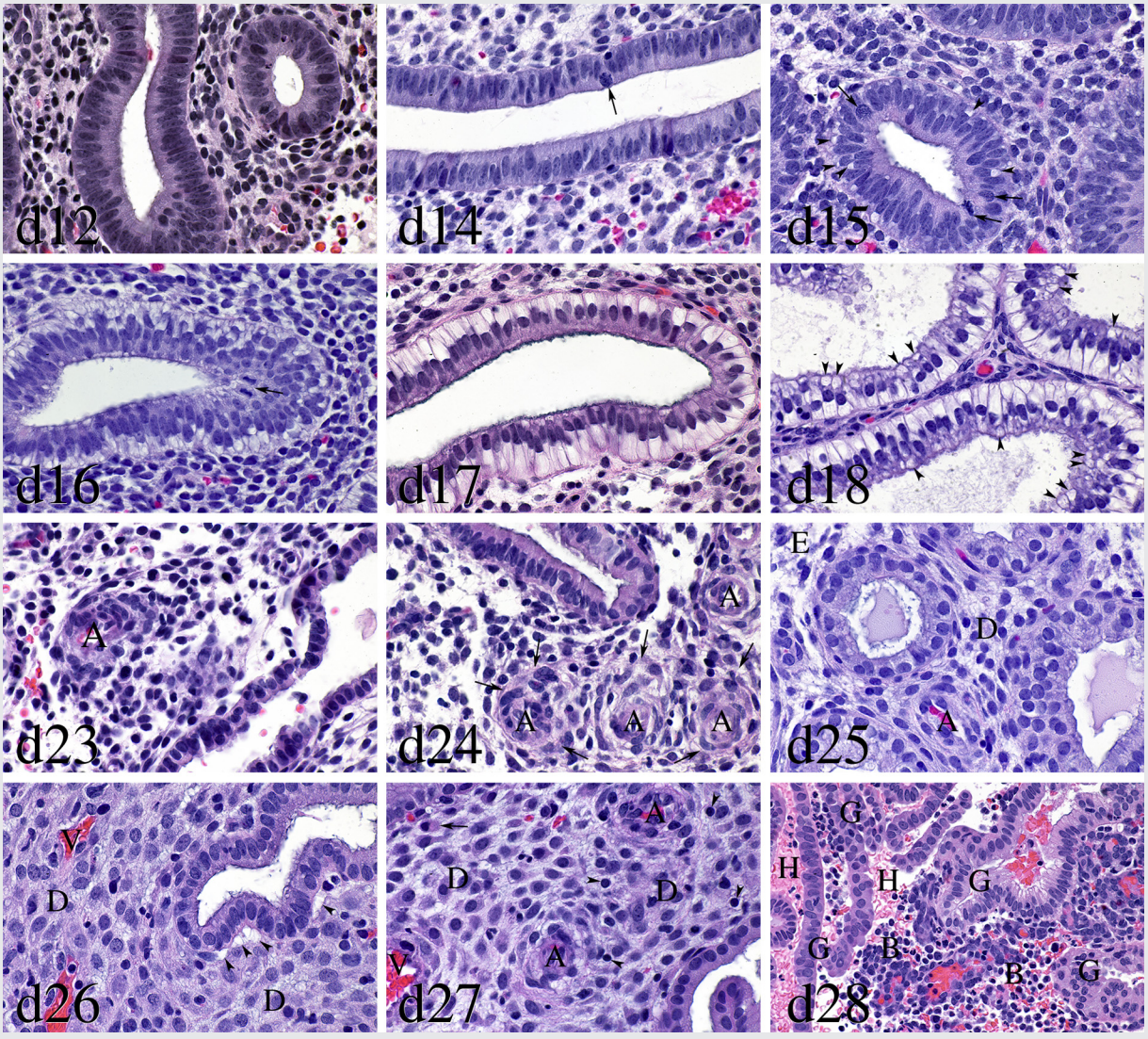
FIGURE 3



Histologic changes of the endometrium through an idealized 28 day menstrual cycle (19, 20). The endometrium changes throughout the menstrual cycle. Endometrial glands appear straight through CD 8, then begin to coil beginning on CD 9. By cycle day 12, the glands are clearly bent (d12), with no evidence of secretions or mitotic figures. On CD 14 (d14)—the day of ovulation—the first mitotic figures are noted (*arrow*), while secretory vacuoles remain absent. On CD 15, the first subnuclear vacuoles can be appreciated (*arrow heads*) involving less than 50% of the glands. Mitotic figures become more numerous (*arrows*). Greater than 50% of the glands exhibit subnuclear vacuoles on CD 16 (d16), which are so numerous that the glandular nuclei appear pseudostratified. Mitotic figures are now frequent (note daughter cells, *arrow*). On CD 17 (d17), the subnuclear vacuoles are evenly aligned leading to a uniform row of nuclei. On CD 18 (d18) the secretory vacuoles move to assume a luminal position (*arrow heads*). Mitotic figures are now only rarely seen. Between CD 19 and 22 the glands first lose their vacuoles and then the stroma becomes progressively edematous. On CD 23 (d23) a clear marker appears, the presence of prominent spiral arterioles (A). By CD 24 (d24), cuffs of decidualized stromal cells (*arrows*) appear to wrap around the spiral arterioles (A).CD 25 (d25) is characterized by an even amount of edematous (E) and decidualized (D) stroma, which surrounds the spiral arterioles (A). The decidualization of the stroma continues until CD 26 (d26) when almost the entire stroma is a continuous sheet of decidualized cells (D), with only a few areas of edema remaining (*arrow heads*). By CD 27 (d27) the stroma is completely decidualized (D), with interspersed spiral arterioles (A) and veins (V). In addition to decidualized stromal cells, lymphocytes (*arrow heads*) and large granulated lymphocytes (*arrow*) can be seen infiltrating in and around the stromal cells. On CD 28 (d28), the stroma has broken down (B), with obvious areas of hemorrhage (H) and interspersed glands and gland fragments (G).

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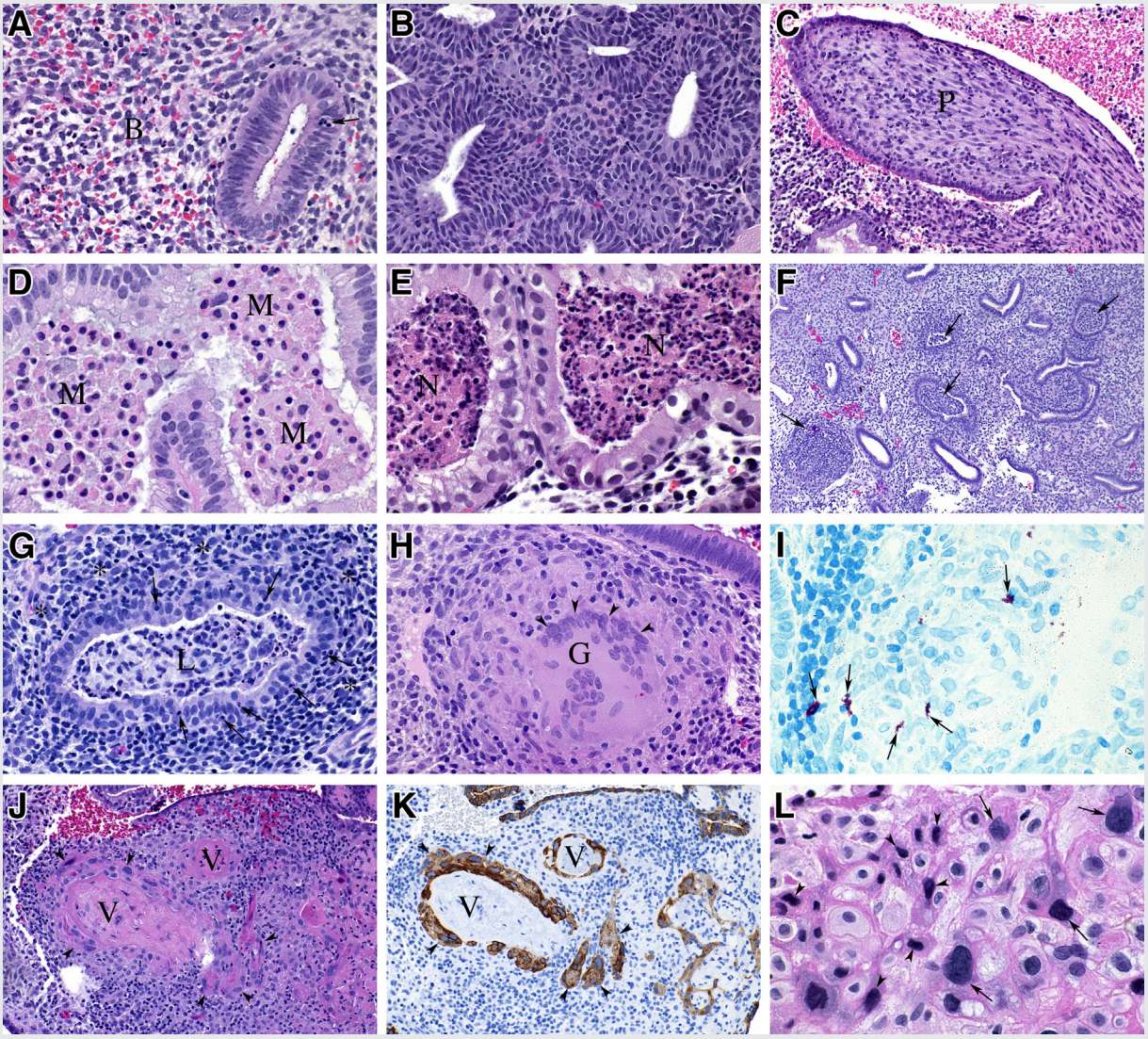
responsive endometrial glands. However, even in the presence of adequate or supraphysiologic levels of E and P, endometrial glands may not be responsive (inactive glands) (Fig. 4A). The glands may not grow in the presence of E and/or they may not differentiate and become secretory in the presence of P. Such an endometrium neither allows for implantation nor placentation. In the case of unresponsive endometrial glands, the route and dose of exogenous hormones should be investigated.

FIGURE 4



Representative pathologic conditions seen in endometrial biopsies. **(A)** A day 24 biopsy reveals inactive endometrial glands with evidence of apoptotic figures (*arrow*) and breakdown of the stroma (B) with extravasated erythrocytes and neutrophils. **(B)** A day 15 biopsy reveals piled up endometrial glands with almost no intervening endometrial stroma, consistent with complex hyperplasia without atypia. **(C)** An endometrial polyp with lymphocytes infiltrating the polyp's stromal core (P). **(D)** The gland lumen of a day 24 endometrium filled with macrophages (M), a common finding in cases of endometriosis. **(E)** Gland lumens filled with neutrophils and cellular debris from a day 24 endometrial biopsy diagnostic of acute endometritis. **(F)** A low-power view of a day 25 endometrial biopsy with evidence of diffuse infiltration of the endometrial glands by a mixture of lymphocytes and macrophages (*arrows*). **(G)** Higher-power image of specimen shown in **(F)** reveals an endometrial gland filled with lymphocytes (not plasma cells) and macrophages (L). The gland is also surrounded by a dense lymphocytic infiltrate (*), which are not plasma cells. Lymphocytes can be seen infiltrating the glandular epithelium (*arrows*), suggesting a glandular destructive process. **(H)** Granuloma within the endometrial stroma with multinucleated giant cells with nuclei arranged like a horseshoe (*arrow heads*) (Langhans giant cell) often seen in cases of tuberculosis. **(I)** Acid-fast stain of granuloma seen in (H) with obvious red acid-fast bacilli (*arrows*) consistent with tuberculosis. **(J)** Foci of hyalinized cells (V) surrounded by large cells with hyperchromatic nuclei (*arrow heads*), suggestive of degenerating retained chorionic villi. **(K)** Low-molecular weight cytokeratin immunohistochemistry of the same area shown in **(J)** confirms retained products of conception, with villous profiles (V), surrounded by trophoblasts (*arrow heads*). **(L)** Focus of hyperchromatic, anaplastic trophoblasts some with very large (*arrows*) and some with smaller nuclei (*arrow heads*) consistent with a placenta site trophoblastic tumor.

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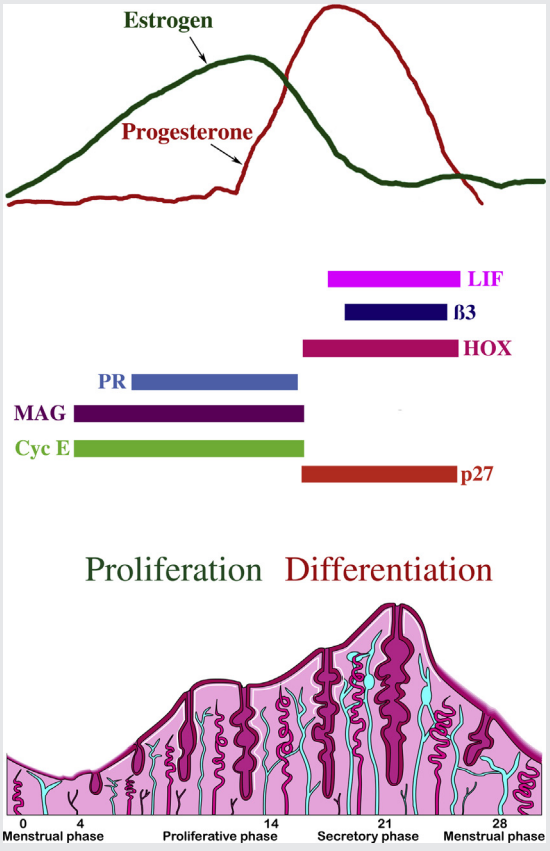
On the opposite extreme endometrial glands can be seen to be highly proliferative even in the absence of exogenous E stimulation (hyperplasia) (Fig. 4B). With hyperplasia, treatment should focus on arresting excessively proliferating glands before further treatment with E is contemplated as hyperplasia can progress to endometrial glandular carcinoma. Endometrial polyps (Fig. 4C) represent another endometrial growth disorder that may result from a hyperestrogenic state (26). When observed, polypectomy prior to embryo transfer should be considered.

FIGURE 5



Markers of endometrial development. Estrogen induces endometrial proliferation, while progesterone blocks proliferation and induces endometrial differentiation (*top* and *bottom*). Markers of endometrial development (*middle*) logically segregate between the proliferative and differentiation phases of the menstrual cycle as the specialized products made in each phase are most often distinct. Cyclin E (Cyc E, the regulator of the Gap 1 to S phase transition of the mitotic cycle); p27 (the specific inhibitor of cyclin E); mouse ascites golgi (MAG; a large mucin found in Blood group A and AB patients); progesterone receptor (PR, up-regulated by estrogen and down-regulated by progesterone); homeobox protein HOXA10 (HOX, an endometrial transcription factor); α_v/β_3 integrin (ß3; binding partner of fibronectin); leukemia inhibitory factor (LIF; promotes cell differentiation).

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Inflammatory Lesions

A variety of inflammatory lesions can be identified histologically. Glandular lumen macrophages (Fig. 4D) are a possible marker of the pelvic inflammation seen in endometriosis. Glandular lumen neutrophils (Fig. 4E), a marker of a frank bacterial infection necessitates antibiotics. A less common, and enigmatic, inflammatory reaction is characterized by lymphocytic infiltration around, through, and in the glands by a mixture of lymphocytes and macrophages (Fig. 5F and G). This is not "chronic endometritis" which is characterized by a few sporadic plasma cell-like stromal cells (27). Rather, it is an actively destructive process that may represent either an immunologic response to auto-antigens (28, 29) or possibly a viral infection with an agent such as HHV-6a (30). Tuberculosis is heralded by the presence of classic granulomas (Fig. 4H) with an associated positive acid-fast stain (Fig. 4I).

Retained Products of Conception

Finally, evidence of retained products of conception can often be identified by standard hemotoxylin and eosin staining (Fig. 4J) and confirmed by cytokeratin staining of residual villous and invasive trophoblasts (Fig. 4K). The presence of persistent trophoblasts within the endometrium can interfere with normal endometrial development and lead to implantation failure. Foci of retained trophoblasts can rarely progress to potentially lethal placental site trophoblastic tumors (Fig. 4L) (31). None of these aforementioned pathologic conditions can be assuredly diagnosed by molecular means that do not preserve endometrial architecture. Avoiding histologic examination of the endometrial biopsy prevents the diagnoses of these conditions, precludes appropriate treatment, and may therefore be life threatening.

However, histopathology alone is not sufficient to assess the functional state of the endometrium. The need to better assess endometrial function and dysfunction led to the identification of markers that may indicate the functional state of the endometrium.

CANDIDATE MARKERS FOR ENDOMETRIAL ASSESSMENT

As discussed above, the endometrium is a uniquely dynamic mixture of tissues that undergoes a continuous process of proliferation, differentiation, destruction, and renewal. The drivers of these transformations are ovarian derived estrogen and progesterone. At the simplest level, estrogen drives endometrial proliferation, followed by progesterone mediated differentiation (Fig. 5). Based on this proliferation/differentiation dichotomy, it is not surprising that the most useful markers of endometrial function segregated into either one of these two menstrual cycle phases (Fig. 5). When a marker is present at an inappropriate time of the cycle the development of that endometrium is presumed to be abnormal (32–39).

In cases of unexplained recurrent implantation failure, all the markers point to the same fundamental abnormality: an uncoupling of stromal and glandular development. While the endometrial stroma appears almost impervious to disruption, the endometrial glands are highly sensitive to perturbations, especially delays in development (40–43). The common pattern seen, irrespective of the marker examined, is a stroma matching cycle dating, with glandular development lagging far behind—commonly called glandular developmental arrest (GDA) (40, 44). GDA is a marker of the delayed endometrial development described by Lessey et al. (44) as a Type I implantation defect where the window of implantation is delayed. Correlating this to H&E histologic assessment, a CD 24 specimen from a normal fertile control would show decidual cuffing of the spiral arterioles in the

stroma, and glands with no secretory vacuoles (Fig. 3 d24). Marker wise, progesterone receptor, mouse ascites golgi (MAG), and cyclin E would be absent from the endometrial glandular epithelium, while leukemia inhibitory factor (LIF), α_v/β_3 integrin (ß3), HOXA10 (HOX), and p27 would be seen (Fig. 5). In contrast, an endometrial biopsy from a women with repeated implantation failures would typically show the same decidual cuffing of the spiral arterioles with histologic assessment—in other words, the dating would still be cycle day 24—but MAG and cyclin E would be present in the glands, while LIF, ß3, HOX-A10 and p27 would either be absent or significantly decreased owing to GDA at cycle day 18-9 (32–34,44). In cases of GDA, MAG, and cyclin E would go from no expression to increased expression in a CD 24 specimen, while LIF, ß3, HOX, and p27 would go from high to low expression. As a practical matter it is easier to see the appearance of a marker than its disappearance, making MAG and cyclin E, more sensitive tools to identify GDA.

The behavior of these specific markers helps to explain why endometrial dating by itself is not adequate to diagnose developmental abnormalities in the endometrium (24). A finding echoed by an analysis of 4,526 endometrial biopsy samples from one of the author's (H.J.K.) clinical cases. This demonstrated that dating itself predicted abnormalities in markers of endometrial development with a positive predictive value of 54.8% (95% confidence interval [CI] 52.7, 56.9)—just better than a coin toss.

As the treatment of infertility has evolved, so has the demand for a better understanding of endometrial function and the need for markers of normal and abnormal endometrial development. We review below the clinical needs that have driven these endometrial function tests, what they each teach us, and how we can best utilize these tests to offer the best care for our patients.

ROLE OF ENDOMETRIAL TESTING IN CLINICAL PRACTICE

The process of implantation relies on the presence of a competent embryo and a receptive endometrium (see Fig. 3 in Lessey et al. [44]). Insight into embryo competency began with morphological scoring and culminated in extended embryo culture with preimplantation genetic testing for aneuploidy (PGT-A) (45). In the past, in vitro fertilization embryo transfer) was largely a treatment approach that included controlled ovarian hyperstimulation (COH), fertilization by insemination or intracytoplasmic sperm injection, a short course of embryo culture, and the transfer of multiple embryos. It was the rare cycle in which embryos were not transferred shortly after retrieval. As such, the endometrium was largely at the mercy of COH derived supraphysiologic and accelerated estradiol and progesterone exposure. The constraints imposed by COH on the endometrium made endometrial testing implausible. Endometrial sampling during a transfer cycle is not encouraged (46) and the hormonal milieu seen with COH cannot be altered to effectively manipulate the maturation process of the endometrium.

Advances in embryo culture and freezing have changed the equation for endometrial assessment. Introduced into in vitro fertilization in the late 1990s, extended embryo culture to the blastocyst stage became routine by the mid-2000s and has been shown to result in higher clinical pregnancy and live birth rates than cleavage stage transfers (47). Blastocyst embryo culture enabled better selection of competent embryos, thereby reducing the number of embryos transferred in fresh cycles. It also afforded the opportunity to preserve high quality embryos. This was enhanced with the application of vitrification and PGT-A (48). By 2015, the number of embryo freeze thaw cycles nearly matched the number of oocyte retrieval cycles (49). The transfer of a single competent embryo in a frozen embry transfer is increasingly becoming the norm for women undergoing assisted reproduction technology (50, 51). Implantation failure in this context has shifted the focus from the embryo to the endometrium.

Currently the three commercially available tests for endometrial receptivity employ three different approaches: identifying errors in dating, i.e. synchrony between the embryo and endometrium; identifying errors in function; and identifying inflammatory factors that may interfere with endometrial function. Each of these three approaches requires endometrial sampling at a prescribed time in the cycle. What may seem a simple matter, that of defining what cycle day a particular patient needs to be biopsied, is not. The timing of biopsy depends on whether one considers a natural versus a medicated cycle. Indeed, the variation in preparation regimens—i.e., non-agonist, agonist, artificially triggered natural, and progesterone supplemented natural regimens, to name a few—have led to a confusion of terminology and definitions. Therefore, we have attempted to synchronize the endometrial dating lexicon in Figure 1. We created this figure by first aligning known and agreed upon key menstrual cycle parameters: the day of the luteinizing hormone (LH) surge, the day of ovulation, embryo age, and the progesterone start date—and then added the standard embryo transfer dates and recommended biopsy dates for the three commercially available endometrial assessment tests: the Endometrial Receptivity Analysis (ERA), the Endometrial Function Test (EFT), and the ReceptivaDx test.

Molecular Dating Test: Endometrial Receptivity Analysis

The Endometrial Receptivity Analysis (ERA) is a molecular based test designed to date the endometrium. The proponents of the ERA claim that optimizing the chance that the endometrium is exactly on cycle day 20 will improve implantation rates (52–54). To achieve this accuracy the ERA requires an endometrial biopsy on LH+7 in a natural cycle or P+5 in a hormone replacement cycle [after 5 full days (120 hours) of P] (Fig. 1). As noted in Figure 1, there appears to be an inconsistency of these biopsy dates with a different biopsy day in a natural versus a medicated cycle.

Because the ERA begins by dissolving the biopsy specimen for molecular dating analysis, there is no histologic assessment of the patient's uterine lining. Consequently, the

ERA cannot test for the presence of the pathologic conditions discussed previously (Fig. 4).

In essence, the ERA is a molecular based means of dating the endometrium. As was discussed above, the Cooperative Reproductive Medicine Network determined that dating alone was unable to discriminate between fertile and infertile women (24). Putting this issue aside, there has also been concern raised about the ability of the ERA to date an endometrial sample when compared to classic Noyes criteria (55). These authors also found that only 50% of the patients with an ERA certified receptive test result (ie, had a molecular dating consistent with cycle day 20) conceived in their subsequent FET cycle; and of those patients with pre- and post-receptive ERA results who underwent appropriate adjustment, only 33.3% conceived—which was similar to the 35.2% background ongoing pregnancy rate in the women who had no endometrial testing. On the other hand, other investigators have found utilization of the ERA was associated with improved implantation and pregnancy rates, albeit the differences were not statistically significant (56). It is also not clear why such precision in timing of embryo transfer is critical given that the implantation window is at least three days in duration (2, 3, 57, 58). Given that the human embryo does not experience a diapause (59), narrowing the window or focusing on a precise time of transfer may not be beneficial (58).

Attempts are underway from different groups to enhance the utility of molecular array testing by including markers specific to endometrial glands (60) and maternal immune implantation response markers (61). These second generation molecular array tests may both improve the accuracy of the dating assessment and identify patients that may benefit from specific interventions. However, the same caveat regarding the lack of histologic assessment described above also exists with these tests.

Function Test: Endometrial Function Test

The Endometrial Function Test (EFT) has two components: 1) a histologic assessment, and 2) an assessment of endometrial development. Histologic examination of CD 15 and 24 biopsies for dating is followed by an evaluation for pathologies that are known to interfere with endometrial development and implantation (Fig. 4). As was described above, the menstrual cycle is made up of a proliferative phase, followed by a differentiation phase (Fig. 5). Although many markers have been proposed to assess these two phases, the foundation that all these markers rest upon is the mitotic cycle machinery. This is because cells either proliferate or differentiate—and they cannot differentiate until they stop proliferating. An endometrium that is persistently proliferative cannot manufacture the cellular components that are necessary for blastocyst attachment and implantation. Therefore, the EFT was designed to determine the developmental state of the endometrial by quantitative immunohistochemical assessment of a molecular marker of proliferation (cyclin E) and a marker that stops proliferation (p27)—as described by Dubowy et al. (32).

If an endometrial sample from CD 24 still exhibits glandular cyclin E, i.e., the glands are still proliferating, then that endometrium cannot be receptive to blastocyst attachment and implantation. Such glands are delayed even though the stroma has reached cycle day 24—the definition of glandular developmental arrest (GDA). This is much like a surfer (the glands) who cannot catch a wave (the stroma) because the wave is going too fast.

By examining a panel of markers in a patient's endometrial glands on cycle days 15 and 24 the developmental trajectory between these days can be deduced. For example, if a women's endometrium has developed normally between days 15 and 24, then her endometrium will also be developmentally normal as it passes through the window of receptivity. If, on the other hand, her day 15 sample shows too potent a progesterone response and her day 24 biopsy shows GDA, then adjustments can be made to her stimulation protocol to correct these abnormalities. This approach was validated by observing that women who had an abnormal EFT with no form of intervention were 10.5 times less likely to have an ongoing pregnancy (odds ratio 10.5, 95% CI 1.29, 680; positive predictive value = 91%, 95% CI 72, 100; $P<.001$; Fisher Exact Test) compared to women who had a normal EFT or had an intervention following an abnormal EFT (62).

The EFT does have limitations. First, two biopsies are required for the EFT and processing of the specimens is labor intensive and time consuming. Second, an expert reproductive pathologist is needed to assess the slides produced for the test. And, because the results are the product of human interpretation, read-to-read reliability may be a factor. However, a cohort of 100 samples were analyzed repeatedly between 3 and 35 times resulting in an excellent (63) intraclass correlation rating of 0.76 (95% CI 0.70, 0.82) (64). Given these limitations, use of the EFT should be reserved for cases where there are a small number of embryos available for transfer, such as in cases of donor embryo transfers (65), transfers after oocyte cryopreservation (66), or when repletion of embryos is not possible.

Inflammatory Marker Test: ReceptivaDx

The ReceptivaDx test evaluates an endometrial sample for an inflammatory marker associated with endometriosis. This makes biologic sense since endometriosis has been shown to influence implantation potential (67–72). As with the two tests described above, this test also starts with an endometrial biopsy—in this case collected anytime from LH+6 to LH+10 in a natural cycle or P+5 to P+10 in a stimulated cycle (Fig. 1). The ReceptivaDx test does not include a pathologic assessment of the submitted sample, but instead focuses on the immunohistochemical expression of B-cell CLL/lymphoma 6 (BCL6), a marker of endometriosis (73). Almquist et al. (74) compared 17 patients with normal BCL6 expression to 52 women with abnormal (increased) BCL6 expression and found a significant decrease in pregnancy and live birth rates with increased BCL6 expression. These studies imply that treatment for

endometriosis following a positive ReceptivaDx test will improve pregnancy outcomes (67, 75, 76).

SUMMARY

With unexplained implantation failure, the clinician should consider assessing the endometrium. With any cost benefit analysis, as the supply of embryos decreases, their value increases. Once this value reaches a certain point, the benefit of endometrial assessment will outweigh its cost. The more precious an embryo, the more important it is to maximize the chances of that embryo encountering a receptive endometrium.

Because of the endometrium's dynamic nature and critical function, it should not be surprising that its assessment is also complex. This assessment is time consuming, costly, and necessitates an invasive procedure. Therefore a shared decision between patient and clinician should drive when such an assessment is cost effective. This decision requires a discussion of the available tests and which one provides the critical information they need to help achieve a pregnancy. While timing is clearly important, it is not everything (58). The implantation window is dependent upon a normally functioning tissue. Comprehensive testing should include an assessment of dating, screening for pathologic conditions, and the results should give actionable insights into the adequacy of a particular stimulation protocol. In doing so, the clinician can offer the best options for those who have failed multiple transfers with high quality embryos.

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