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Evaluation of endometrial function: a Heraclean or Sisyphean task?

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More than 40% of high-quality, euploid embryos fail to implant or are lost in early pregnancy. These assisted reproductive technology failures, coupled with cases of recurrent implantation failure and unexplained recurrent miscarriage, argue strongly that endometrial receptivity plays a significant role in assisted reproductive technology outcome. In this issue of *Fertility and Sterility* Diaz-Gimeno et al. (1) present an interesting article describing a further refinement of the endometrial receptivity array (ERA) test, which may improve its predictive value for biochemical pregnancy loss and potentially provide better clinical guidance to improve pregnancy outcomes.

Development of accurate and clinically useful measures of endometrial receptivity remains a difficult area of investigation. Attempts to assess endometrial receptivity date back at least to the highly influential article by Noyes, Hertig, and Rock, published in 1950 as the first article in the first issue of this journal (2). The experiences of the intervening 67 years suggest that the quest for a reliable endometrial function test may be described by analogy to either of two well-known figures from Greek mythology: Sisyphus and Heracles. The very clever King Sisyphus was punished by Zeus for his intellectual hubris by being forced to push a large boulder to the top of a steep hill, but the boulder was enchanted to roll away each time, just before reaching the top. Thus was Sisyphus fated to eternally labor without ever reaching his goal. Heracles was given 12 seemingly impossible tasks by King Eurystheus to atone for his misdeeds. In contrast to Sisyphus, Heracles completed all the tasks with a combination of brute force and cleverness. Whether accurate assessment of endometrial receptivity is ultimately a Heraclean or Sysiphean endeavor, the work has been difficult, and the goal has not been completely achieved.

Although the 67-year-old histologic evaluation method of Noyes has been shown to lack clinically sufficient precision and reliability (3), one of the core concepts underlying Noyes's approach remains strongly supported by the accumulated data: continued endometrial exposure to sufficient P determines a temporal "window of implantation" (WOI), a time of optimal receptivity that allows the best outcome for embryo implantation. The ERA and its refinements are based on the related principle that an individual woman's optimal window is temporally shifted or narrowed under some conditions, by unclear mechanisms, so that the time of optimal endometrial receptivity occurs either before or after the time of embryo developmental readiness to implant. As might be expected, some of the key markers of receptivity in the ERA test are genes whose endometrial expression is known to be profoundly regulated by P and/or stromal decidualization (e.g., *PAEP*, *IGFBP1*, and *S100P*).

Clinical application of the ERA test involves “personalization” of the timing of frozen embryo transfer to achieve the best possible synchrony between endometrial and embryonic readiness. Clinical viability of the ERA also requires that individual differences in the WOI are highly consistent from cycle to cycle, at least when using an identical hormonal preparation of the endometrium. Given the long history of the misapplication of the histologic method, it will be important to carefully evaluate the reliability of this newly refined ERA test and the effectiveness of its application. Only then will we understand its strengths and limitations and for whom and how much it provides a clinically significant benefit.

The original ERA test identified prereceptive, receptive, and postreceptive transcriptomic signatures using a custom DNA microarray examining 238 genes, and currently the assay uses an analogous RNA-seq approach examining 236 genes (Igenomix website). In the present study the original microarray method is used, and machine learning algorithms are used to segregate transcriptomic patterns from a series of training sets, resulting in generation of further subtypes of gene expression patterns. The newly defined patterns include a pattern that clusters between prereceptive and receptive, dubbed “late prereceptive,” and a pattern that clusters between receptive and postreceptive, termed “late receptive.” It should be noted that these patterns are not determined by or necessarily correlated with either LH dating or morphologic dating. The interesting finding, however, is that application of these new subclassifications to an independent set of clinical samples from women with previously determined normal ERA findings yields a classification that seems to predict chemical pregnancy loss.

Reassuringly, excellent outcomes are seen in the subjects whose endometrium exhibits a transcriptomic profile consistent with the now more rigorously defined receptive pattern. In addition, patients with the newly defined late prereceptive pattern have outcomes highly similar to that of the receptive pattern, suggesting that the late prereceptive and receptive patterns are functionally equivalent. The potentially most important finding is seen in patients with the newly defined late receptive pattern. Curiously, no difference was seen between receptive and late receptive groups in regard to pregnancy rate (66.6% for late receptive pattern vs. 56.8% for receptive pattern). However, the late receptive pattern was associated with a 7-fold increase over the receptive group in biochemical loss rate! Although the precision of these estimates is strongly tempered by the fact that the late receptive group is small and rare in this study ($n = 9$ of $n = 321$ in the clinical outcomes comparison), these findings bolster support for an endometrial factor in some cases of biochemical pregnancy loss. Diaz-Gimeno et al. compare these results with the classic study of natural conceptions by Wilcox et al. (4), which suggested that late implantation resulted in a high risk of early pregnancy loss, though the Wilcox study also demonstrated that late implantations are quite rare in contrast to the high frequency of implantation in the late receptive pattern described in the present article.

It is also important to note that the vast majority of failed implantations, biochemical losses, and clinical losses were in the normally receptive groups, demonstrating that altered ERA profiles are not seen in the vast majority of failed or abnormal implantations, at least in the population studied. It will be interesting to see the results of such a test applied to euploid

embryos in women with and without recurrent implantation failure, to gain further insight into the causes of these losses. Additionally, a small number of these samples, previously noted to be normal by ERA, are now classified as markedly shifted to postreceptive or early prereceptive, suggesting that the test–retest reliability of an identical sample is good but not perfect. Consistency between cycles should also be explored to determine whether the new subclassifications are stable from cycle to cycle, especially given the small number of subjects on which the claim of cycle to cycle consistency of ERA is based. Finally, if the results hold up with larger numbers of patients with a late receptive pattern, this test may give us important mechanistic insights into one cause of chemical pregnancy loss.

As discussed above, the ERA test is built on a conceptual model that suggests alteration of timing and/or length of the WOI is the most important endometrial determinant of normal implantation. An alternative conceptual model, that inflammation or other pathologies alter endometrial function independent of WOI timing, has led to development of other tests based on one or a small number of functional biomarkers (e.g., ReceptivaDx [disclosure: S.L.Y. is a co-inventor of the intellectual property on which this commercial test is based]). Curiously, one of the RNA markers clearly associated with biochemical loss in the present article is *BCL6*. Our group has identified BCL6 protein overexpression as an important marker of endometrial dysfunction in women with endometriosis (5), suggesting some reassuring overlap between functional candidate protein approaches and those of machine-assisted learning transcriptomic approaches. Continued, careful studies of “personalization” of ET based on the altered window hypothesis vs. treatment of endometriosis, inflammation, or other pathophysiologic states will help to delineate whether either or both of these etiologic hypotheses best describes the cause(s) of implantation failure, biochemical loss, or later complications of abnormal implantation. Although much work remains to be accomplished to understand factors regulating implantation, I have high hopes that the truly laborious efforts of Diaz-Gimeno et al. and other groups will result in a more Heraclean than Sisyphean outcome.

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