Recurrent implantation failure is a pathology with a specific transcriptomic signature

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Recurrent implantation failure (RIF) is a source of distress and frustration to both patients and their clinicians. In the absence of clinically useful tests, the therapeutic approach has been largely empirical, with limited efficacy. In recent years, new insights into the role of the endometrium in implantation have emerged, and a number of dysfunctions that may underlie implantation failure have been characterized. These point to the presence, in some patients, of an underlying endometrial pathology. In this article, the case is made that constitutive (rather than maturation) defects underlying RIF can be identified. Evidence is presented of a specific transcriptomic signature that is highly predictive of RIF. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
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Despite impressive advances in assisted reproductive techniques, and in particular the advent of more effective means of embryo selection and cryostorage, many patients continue to suffer the disappointment of serial IVF failure. This outcome is particularly frustrating to both patients and their caregivers when other parameters of success, such as number and quality of oocytes and embryos, have been encouraging. The lack of evidence-based therapeutic solutions means that clinicians often feel obliged to offer treatments that are largely empirical, based on a degree of plausible biologic rationale but with little clinical evidence base to support their use (1). However, that recurrent implantation failure (RIF) of apparent endometrial origin should be encountered in the context of IVF treatment is not surprising when around one-third of couples emerge from fertility investigations with no identified cause for their predicament. "Unexplained infertility" is so common that it has gained a certain shrugging acceptance in the consultation room, supported in part by the efficacy of assisted reproductive technologies (ART) in addressing the unknown underlying problem. It does, however, represent an unmet need in our field: the lack of useful tests to assess the function of one of the key pillars of reproductive success, i.e., the healthy endometrium. In vivo periimplantation events remain in a "black box" (2), continuing to elude our full understanding.

RIF remains a variably defined condition, but the absence of implantation after three or more transfers of high-quality embryos or after placement of ten or more embryos in multiple transfers have been proposed as criteria to identify patients who might benefit from further investigation (3, 4). Studies of the probability of a systemic underlying cause for implantation failure in these patients, rather than simply a chance effect, indicate that an underlying etiology for both their experience of RIF and indeed their infertility is likely to exist in many patients (5, 6). Multiple causes of implantation failure have been proposed, but until recently the primary focus has been on the embryo, and in particular the impact of aneuploidy (7). Maternal factors may also contribute, and the clinical approach to investigating RIF now involves the exclusion of thrombophilic gene mutations, autoimmune conditions, and uterine anomalies. However, in the majority of cases, no clear cause can be identified (8, 9). In recent years it has become apparent that constitutive endometrial dysfunction could represent an important contributor to this condition and to infertility in general (10, 11).

RECURRENT IMPLANTATION FAILURE REPRESENTS MORE THAN AYSNCHRONY

The concept of the window of implantation that emerged from studies of laboratory animals gained more credence as a feature of human reproduction with the classic studies of Wilcox et al. relating implantation outcome with its timing in relation to ovulation

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(12). However, until recently the only means of assessing this was by histologic examination of timed endometrial biopsies and application of the "Noyes" criteria for dating endometrial maturation (13). In the absence of any objective means of assessing endometrial receptivity, the Noyes criteria became widely used, and the concept of endometrial dysfunction being rooted simply in degree of secretory advancement gained currency. However, whereas the Noyes criteria for the histologic assessment of endometrial tissue were originally designed only to enable the number of days of endometrial exposure to progesterone to be assessed, they became considered to represent a measure of endometrial receptivity in general. It soon became clear that dating the maturation of the secretory endometrium was a poor predictor of implantation (14). Although this failure was blamed largely on the subjective nature of histologic assessment, it has since become evident that endometrial receptivity is determined by more factors than simply an appropriate maturation response to progesterone (5). The widespread and effective practice of transferring day 2 human embryos into the uterus, in the knowledge that they can "wait" for the endometrium to become receptive suggests a level of embryo tolerance of the nonreceptive endometrium. It can be argued, therefore, that encountering perfectly synchronized endometrium is not mandatory for successful embryo implantation.

Although endometrial gene expression has been shown to be sensitive to cyclical hormonal regulation and exogenous hormonal treatments (15–18), a number of studies have shown other factors to affect endometrial gene transcription, which is altered in the presence of gynecologic pathologies, such as endometriosis (19) and during the use of an intrauterine device (20). It is therefore reasonable to conclude that although asynchrony may be the cause in some (21), RIF may also arise as a result of other causes and that these may include constitutive disruptions of endometrial function. The potential value of identifying a gene expression profile predictive of RIF is considerable, because this would not only guide prognosis, but also could inform appropriate and effective therapeutic intervention.

IS THERE AN ENDOMETRIAL GENE TRANSCRIPTION PROFILE UNDERLYING RECURRENT IMPLANTATION FAILURE?

In recent years, a number of studies have appeared comparing transcriptomic profiles in endometrium from fertile women with those from women with a history of otherwise unexplained RIF (11, 22). However, most of these studies have not been subjected to validation on an independent cohort. Our group therefore sought to investigate in an initial matched cohort study whether an endometrial gene profile could be identified in women with a history of RIF, and then to test the predictive value of this classifier in a further group of patients.

The methodology used is described in detail elsewhere (22). Briefly, two cohorts of patients who had undergone, owing to standard clinical indications, in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) treatment with the use of similar treatment regimens were recruited. The study cohort consisted of ovulatory women who had experienced RIF after undergoing three or more embryo transfer procedures or transfer of ten or more high-quality embryos, despite a good ovarian response and in the absence of uterine pathology. The control cohort included women who had readily conceived after ICSI for male-factor fertility. RIF patients were screened for relevant inherited and acquired thrombophilias and abnormalities in glycated hemoglobin and thyroid-stimulating hormone levels, and those demonstrating abnormal results were excluded.

In total, 43 patients with RIF and 72 control subjects were recruited, and each underwent an endometrial biopsy 7 days after an LH surge in their natural cycle. To identify a gene signature for RIF, a randomly selected subset of samples was created whereby the ratio of RIF patients to control subjects was kept similar to the full complement of samples. The remaining samples were assigned to the validation set in the same ratio of RIF patients to control subjects. Signature discovery consisted of 100 rounds of randomly selecting a training subset from the signature discovery set, which was subsequently used to rank genes based on their potential to differentiate RIF patients from control subjects. Samples were randomly assigned into a signature discovery set ($n = 81$) and an independent validation set ($n = 34$), keeping the ratio of RIF patients to control subjects similar. Iterative rounds of cross-validation were applied within the signature discovery set to find genes capable of distinguishing RIF patients from control subjects, because this reduces the risk of overfitting in the signature discovery set. Each iteration results in a separate gene set, and all genes were ranked according to how frequently they were present in the separate gene sets. Selecting all genes with a frequency of $\geq 5\%$ resulted in a 303-gene signature. To validate the gene signature, a classifier was built with the use of the full signature discovery set as input and the profile used to predict the class of the samples in the validation set, which, to ensure an independent validation, had not been used in any of the previous steps.

The positive predictive value (PPV) of the RIF prediction classifier was 90% with a sensitivity of 90% (Table 1). Most importantly, application to the independent validation set confirmed the signature's ability to distinguish RIF patients from control subjects (Fig. 1). All samples classified as RIF were indeed RIF patients (PPV 100%) with a sensitivity of 58%.

TABLE 1

The accuracy of recurrent implantation failure prediction with the use of a 303-gene classifier.

Metric	Signature discovery	Validation
NPV	94.0 (83.8–97.9)	81.5 (63.3–91.8)
PPV	90.3 (75.1–96.7)	100 (64.6–100)
Sensitivity	90.3 (75.1–96.7)	58.3 (32.0–80.7)
Specificity	94.0 (83.8–97.9)	100 (85.1–100)
Overall accuracy	92.6 (84.8–96.6)	85.3 (69.9–93.6)
P value	3.83×10^{-13}	

Note: Values are presented as % (95% confidence interval). Sensitivity and specificity of *P* was calculated with the use of Fisher exact test (two sided). Reproduced with permission from Koot et al. (22). NPV = negative predictive value; PPV = positive predictive value.

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