Implantation failure of endometrial origin: it is not pathology, but our failure to synchronize the developing embryo with a receptive endometrium

Cecilia T. Valdes, M.D.,[a] Amy Schutt, M.D., M.S.,[a] and Carlos Simon, M.D., Ph.D.[a,b,c,d]

[a] Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas; [b] Department of Obstetrics and Gynecology, Valencia University and INCLIVA, Valencia, Spain; [c] Igenomix, Parc Cientific Valencia University, Valencia, Spain; and [d] Department of Obstetrics and Gynecology, Stanford University, Stanford, California

Repeated implantation failure (RIF) is an intriguing, massive failure of reproductive treatment in otherwise healthy women leading to the introduction of empirical adjuvant interventions that are costly, inefficient, and frustrating for our patients. In this article, we will try to convince the readers that RIF is neither a stigma nor a mysterious pathology but rather our failure to diagnose and properly synchronize the euploid blastocyst with the patient's personalized window of implantation. (Fertil Steril® 2017;108:15–8. ©2017 by American Society for Reproductive Medicine.)

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"Insanity is doing the same thing over and over again expecting different results."

Albert Einstein, 1879–1955

Repeated implantation failure (RIF) is an intriguing clinical quandary in reproductive medicine that remains poorly characterized in otherwise healthy women (1, 2). The direct consequence of our inability to understand the etiology has led to the introduction of numerous empirical and thus far ineffective adjuvant interventions that are costly, inefficient, and frustrating for our patients.

Repeated IVF failure represents an enormous emotional and in some countries financial burden for the patient. Failure to achieve the goal of being a parent is a worldwide public health issue, causing feelings of helplessness, depression, and anxiety in both men and women (3, 4). Despite the strong desire to become a parent, 50% of infertile couples do not seek treatment, and 50%–60% of couples drop out of treatment after failing two or three IVF cycles even when IVF is provided by employer- or government-funded health insurance (5). There are multiple reasons for this failure to use available fertility treatment, but when cost considerations are removed, psychological stress and a poor prognosis are the main reasons for dropping out of treatment (6). We cannot afford repeated failures of implantation because patients have limited financial and psychological resources. Maximization of IVF success using available resources before patients drop out is imperative.

Various definitions of RIF exist, but one expert proposed pathologic implantation failure be defined as failure of three IVF cycles in which one or two high-grade quality embryos were transferred to the patient in each cycle (2) or after two failures in oocyte donor recipients.

For academic reasons, the causes of RIF can be grouped into several categories, the first of which includes pathological alterations of the endometrial cavity such as hyperplasia, submucosal myomas, or endometrial polyps, endometritis, and synechiae (which can be found in 18%–27% of cases) (7). Other categories include hydrosalpinx (8)—either acting through a direct embryo-toxic effect or adversely affecting endometrial receptivity (9)—an increased incidence of embryonic chromosomal abnormalities (10, 11), obesity (12),

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Reprint requests: Carlos Simon, M.D., Ph.D., Igenomix SL, Narcis Monturiol Estarriol, 11, Parque Tecnológico Paterna, Valencia, Spain 46980 (E-mail: carlos.simon@igenomix.com).

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and lifestyle/other causes such as hereditary and acquired thrombophilias (13). A potential immunological factor has been used unsuccessfully in explaining and treating this condition (14).

In clinical practice, all the pathological issues indicated above, if diagnosed, can be and must be corrected. Then finally we will face an intriguing situation involving two necessary collaborators: the embryo and the endometrium. It is obviously critical to ensure the adequacy of the embryo and endometrium individually but of paramount importance to determine ideal timing and synchronization. Timing is everything in life; the first major milestone is at fertilization, and the second is at conception (15).

Our goal here is to convince the reader that RIF in a significant group of patients is not a stigma or a mysterious pathology, but rather our failure in diagnosis and proper synchronization of the euploid blastocyst with the personalized window of implantation (WOI) in each individual patient.

THE EMBRYO

The diagnosis of morphological and/or chromosomal alterations of the embryo in patients with RIF has been investigated. The data on the effect of both embryonic chromosomal analysis using fluorescence in situ hybridization for preimplantation genetic screening (PGS) and assisted hatching in improving clinical outcomes in RIF patients are controversial (16). Two randomized clinical trials (RCTs) performed in RIF patients had conflicting results. No significant differences in clinical pregnancy rates using PGS were found in RIF patients when all ages were included (17). Conversely, in women $<$40 years with three or more failed IVF cycles without other known causal factors, a significant increase in ongoing pregnancy rates per oocyte retrieval (47.9 vs. 27.9; $P=.04$) and ongoing implantation rates (36.6 vs. 22.1; $P=.01$) in the PGS versus the blastocyst group was reported (11). Now new diagnostic technologies (array comparative genomic hybridization or next-generation sequencing) have become available, allowing all 46 chromosomes to be interrogated, and the concept of preimplantation genetic diagnosis for aneuploidy (PGD-A) has become revitalized. Several PGD-A RCTs are ongoing in the United States, and the clinical community particularly in the United States is keen to critically assess the role of chromosomal normalcy of embryos in patients with RIF.

The variable timing at which an individual embryo is ready to implant informs us about individual variability in embryonic maturation. Blastulation is the best surrogate marker available and is maternal age dependent. Two independent groups demonstrated that patients under 30 had much higher blastulation rates before day 6 than those 31–34 and 35–40 (18), and patients age $\geq$35 have a significantly higher proportion of embryos that fail to blastulate by day 5 when compared with patients $\leq$35 (19). In fresh ETs, implantation rates of embryos that blastulate on day 6 versus day 5 are decreased by 15%–18% (20, 21). Interestingly, the cryopreservation and subsequent transfer in a synchronous programmed endometrium of late blastulating embryos restore their implantation capacity (19, 20, 22). These data strongly suggest that the adverse clinical outcomes of embryos that blastulate late in fresh IVF cycles are due, in large part, to dysynchrony and not to an unknown embryonic abnormality. A previous study from our group found that the personalized endometrial WOI does not change in the individual patient (23), suggesting that the impact of blastocyst dysynchrony may be greater in older women, together with the known increase in embryo chromosomal abnormalities (24).

THE ENDOMETRIUM

The mucosal layer of the uterus is the anatomic prerequisite for survival of the species in mammals and the main target of cyclicity that is driven by the ovary in menstruating species such as humans. This highly dynamic tissue undergoes cyclic cellular proliferation, differentiation, and immune cell trafficking in response to changing circulating ovarian-derived E_2 and P_4 and, in the absence of pregnancy, tissue breakdown, followed by regeneration. The main objective of this complex process is successful adhesion, invasion, and placentation of the conceptus for 9 months followed by involution and subsequent endometrial regeneration postpartum.

The concept of endometrial receptivity and the existence of a window of opportunity were first suggested by Hertig and Rock in 1956 (25). In the 1990s, using the ovum donation model, the clinical WOI, which refers to the self-limited period of time in which the embryo must be transferred back to the receptive endometrium, was demonstrated (26). Further work by Wilcox et al. in 1999 (27) popularized the concept that the human embryo implants 8–10 days after ovulation. However, at that time ovulation was identified on the basis of changes in urinary excretion of estrone 3-glucuronide and pregnanediol 3-glucuronide, which were measured by radioimmunoassay. Now, 17 years later, the above method proposed by Wilcox et al. to determine ovulation has not been clinically adopted and we also recognize limitations of the use of LH measurements in urine or even in blood to predict ovulation (28). Nevertheless, the clinical community has since extrapolated these data to mean that the endometrium in all patients becomes receptive during a wide time frame, 8–10 days after ovulation, with the same success during these 3 days regardless of individual variations or hormonal treatment received (natural cycles, controlled ovarian stimulation, hormone replacement cycles).

Unlike the embryo, the transition from anatomical to molecular medicine in the diagnosis of endometrial function just happened a decade ago. Pioneering work demonstrates the feasibility of the molecular classification of the endometrium using transcriptomic profiling throughout the menstrual cycle (29, 30), as well as during the window of receptivity or WOI (31). Accumulating evidence has demonstrated that we are ready for primetime in the molecular diagnosis of endometrial function (for review, see reference [32]). Our group identified the transcriptomic signature of endometrial receptivity composed by 238 genes leading to the creation of the endometrial receptivity analysis (ERA) (33). The ERA is now performed by next-generation sequencing that is coupled with a computational predictor and algorithm able to identify the receptivity of an endometrial sample, providing the personalized WOI (pWOI) of a given patient regardless of its histological appearance (23).

DOES RIF REFLECT ENDOMETRIAL DYSFUNCTION OR SIMPLY DESYNCHRONIZATION BETWEEN THE DEVELOPING EMBRYO AND THE RECEPTIVE ENDOMETRIUM?

Accumulated evidence has suggested that there is an alteration of endometrial receptivity in patients with unexplained RIF. In the pre-IVF era, classical histologic/morphometric analysis revealed that women repeatedly failing donor insemination have altered endometrial progression in relation to their menstrual cycle, suggesting the importance of the endometrium when all other factors are controlled (34). Current transcriptomic studies have demonstrated the dysregulation of 63 transcripts in the endometrium in women with RIF compared with fertile controls (35). Additionally, 313 genes are differentially expressed in endometrial samples collected on day 21 of the cycle in RIF versus fertile women (36), and the identification and validation of a 303-gene signature that predicts RIF has been suggested (37). An in vitro study also demonstrated differential hormonal regulation of endometrial genes in RIF versus controls who became pregnant after IVF treatment (38). Finally, aberrant endometrial prostaglandin synthesis has been reported in patients with RIF (39).

The initial proof of concept that RIF is not an endometrial dysfunction that will stigmatize a patient forever, but rather a desynchronization between embryo and endometrium, was presented in a prospective study demonstrating that the WOI was displaced in 25.9% of RIF patients versus 12% in control non-RIF patients (40). The identification of the pWOI of the RIF patients has led to a new and interesting finding. One in four RIF patients have a displaced/asynchronous WOI, and our computational predictor classified them as nonreceptive endometrium, either pre- (84%) or postreceptive (16%), which was further verified by a second ERA test. Taking this forward, we translated these genomic results to the clinic by transferring embryo(s) according to the pWOI of the patient, providing a "personalized ET" (pET) resulting in a 50.0% pregnancy rate and 38.5% implantation rate, similar to controls. These results suggest that rescue of nonreceptive RIF patients by pET results in normalized pregnancy and implantation rates (40). This initial study has been further validated by the report of a clinical case of successful pET after seven previous failed IVF attempts (four with her own oocytes and three with oocyte donation) (41). This case report was complemented by a pilot study of 17 patients undergoing oocyte donation who had multiple failed implantations with routine ET but were subsequently treated with pET after the diagnosis of their pWOI, resulting in normalization of their reproductive outcome (41). Given these results, we must pose the question of whether RIF of endometrial origin is a "disease" or simply results from our inadequate timing of ET when the individual woman's endometrium is receptive?

An excellent comparative endometrial gene expression analysis between women with RIF and controls reported the identification and validation of a 303-gene signature that predicts RIF with high positive predictive value of 81% and negative predictive value of 81% (37). To produce their endometrial RIF signature, Macklon's group (Koot et al.) applied correction factors to remove transcriptomic variations throughout the variable timing in which the endometrial samples were obtained (LH+5 to LH+8) as their target was the RIF pathology assuming that the WOI was uniform and lasts 4 days (37). These differences are exactly what the ERA test has been prepared to determine, which is crucial to understand physiological personal variations. Other transcriptomic studies have demonstrated different endometrial expression profiles in RIF versus fertile controls on specific days of the cycle (35, 36), although again this could be explained by the fact that RIF patients frequently have displacement of the WOI (40, 41). Furthermore, the fact that pET guided by the individualized endometrial window of receptivity is able to normalize reproductive results in RIF patients versus controls places further emphasis on the relevance of the pWOI using an objective molecular diagnostic tool (33, 40). This exciting finding is currently being studied not only in RIF patients but also during the initial infertility patient evaluation. This prospective multicenter trial investigates differences in implantation, pregnancy, ongoing pregnancy rates, and delivery among women having blastocyst transfer in their first IVF cycle. Patients were randomized to fresh cycles, deferred ET, or pET, and data from the interim study demonstrate a significant increase in pregnancy rates of 25% in the pET group versus the other two, an increase in ongoing pregnancy rates of 11% ($P=.24$), and implantation rates of 12% and 6.4% higher versus fresh and deferred ET, respectively ($P=.21$) (42). Patient recruitment is complete, and final results in terms of live-birth rates will be obtained and published within a year to determine the utility of this endometrial diagnostic intervention in reproductive care.

Given the enormous burden that childlessness represents for our patients, we should strive to use all available tools including endometrial assessment to accomplish the natural human goal of becoming a parent. We have an opportunity to use modern molecular genetic diagnostic techniques to remove some of the "art" from ART. Specifically, timing transfer of an embryo using data derived from the patient's own endometrial transcriptome holds promise and the beauty of more reliable success. It's time has come.

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