ERPeak℠ Endometrial Receptivity Test

Optimizing implantation with precision embryo transfer

Highlights

- Asynchrony between the timing of embryo transfer and the timing of a patient's window of implantation (which has been shown to vary between women) is likely to result in implantation failure
- The ERPeak℠ endometrial receptivity test assists in personalizing the timing of embryo transfer to improve the likelihood of a successful implantation
- ERPeak℠ endometrial receptivity test is a genomic test
  - » Highly sensitive and accurate
  - » Based on validated gene expression profiles
  - » Results benchmarked in a non-inferiority study
- User-independent answers
  - » Advanced statistical models and optimized analysis provide user-independent results
  - » Results provide individualized guide for embryo transfer
- Recommended for patients with recurrent implantation failure and donor oocyte recipients

Introduction

The endometrium undergoes cyclic tissue remodeling during the menstrual cycle, moving through proliferative and secretory phases to synchronize its physiology with ovarian function. In humans, the endometrium is most receptive to implantation of an embryo during a brief period in the mid-secretory phase. This period is known as the window of implantation (WOI), occurring between Days 19 and 21 of the natural menstrual cycle.[1] In any other phase of the menstrual cycle, the endometrium is unreceptive to pregnancy.[2] Therefore, successful implantation requires not only a viable embryo and a receptive endometrium, but also synchrony between the two.[3]

Lack of synchronization between an embryo that is competent to implant, and the timing of endometrial receptivity is one cause of recurrent implantation failure (RIF). Therefore, the correct identification and prediction of the timing of endometrial receptivity is essential for maximizing the effectiveness of assisted reproduction treatments. Indeed, studies have shown that optimizing the timing of embryo transfer can yield a significant improvement in pregnancy rates, with rates of up to 73% observed in patients with RIF who underwent endometrial receptivity testing.[4]

History of endometrial receptivity testing

Interest in endometrial receptivity has existed for several decades[5] and was first investigated using histological analysis for endometrial dating, though this was found to be of limited accuracy.[6] It has been demonstrated that – as is the case in most tissues in the body – the physiological changes that occur in the endometrium happen as a result of differential gene transcription. Over the course of the natural menstrual cycle certain hormone-regulated genes are up- or downregulated and the relative abundance and composition of these gene transcripts at a given time point provides a specific gene signature. By interrogating the gene signature of an endometrial biopsy, it is possible to accurately estimate the physiological timing of that patient's WOI.[4,7]

Technologies used to measure gene signatures

Gene expression has a very wide dynamic range, often with five orders of magnitude between the highest and lowest expressed genes. This is very different from chromosome counting, which has a very limited dynamic range. Various methods for accurately measuring the gene expression within a given sample have been published, including qPCR and NGS. It is well documented that RT-qPCR has been shown to have the widest dynamic range, the lowest quantification limits and the least biased results.[8]

Here we present a high-throughput RT-qPCR platform for the accurate and reliable measurement of gene expression and specific transcriptomic profile identification in an endometrial sample – the ERPeak℠ endometrial receptivity test.

ERPeak[SM] endometrial receptivity test

The ERPeak[SM] test is a genomic test that analyzes the gene expression profile of an endometrial sample at the expected time of embryo transfer (e.g. 5 days after progesterone administration; P+5). The ability to identify what stage of receptivity the endometrium is in at this time allows IVF clinicians the opportunity to adjust the timing of embryo transfer in a subsequent cycle to accommodate for any potential displacement of the WOI.

Development and validation of the ERPeak[SM] test

Gene candidate identification (Figure 1)

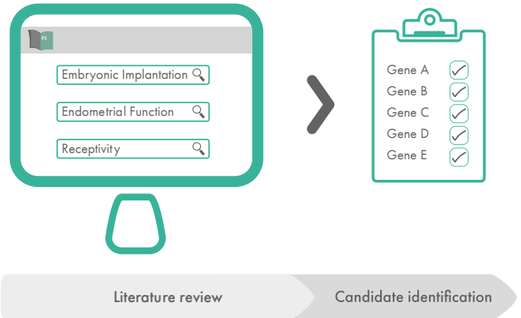
Candidate genes were identified through a thorough review of the scientific literature and online databases, and potential gene functions were assessed on DAVID (the Database for Annotation and Integrated Discovery).[9] Using this approach, 184 candidate genes were identified as potentially informative.[10]

Gene panel selection (Figure 2)

To further refine the gene panel and assess the efficacy of RT-qPCR for endometrial receptivity, a fully consented, two-arm study comparing the gene expression profiles of healthy donors and sub-fertile patients undergoing hormone replacement therapy was conducted.

The healthy female donors (Group A; n=96) had biopsies taken at LH+2 and LH+7 and the sub-fertile patients (Group B; n=120) had biopsies taken at P+5.[10] Analysis of these endometrial biopsies showed that of the 184 genes identified earlier, 85 showed significant differences in fold change between the study groups, and these were selected for further analysis.

Figure 1: Gene Candidate Identification



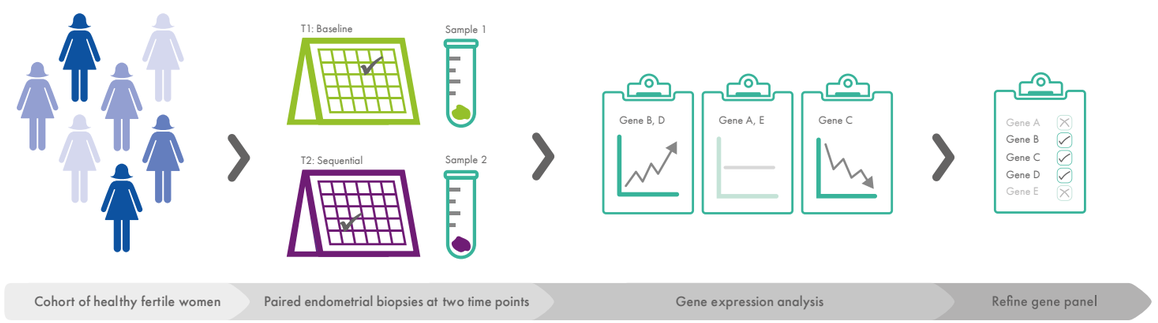
Further analysis of these genes using gene function prediction analysis, known as gene ontology,[11] revealed that all 85 genes were related to cell division and proliferation, cell signaling and response, extracellular organization and communication, immunological activity, vascular proliferation, blood pressure regulation and embryo implantation, further showing their likelihood to be directly involved in endometrial receptivity.

To further understand the key gene signatures involved, principle component analysis (PCA) of the 85 genes was carried out, revealing that a subset of genes explained more than 99.5% of total sample variance in estimating the receptivity status of the endometrium.[10]

Non-inferiority study (Figure 3)

To assess the performance of the ERPeak[SM] endometrial receptivity test, an IRB exempt research protocol was written to conduct a non-inferiority study to compare results to the current clinically accepted assay. Eligibile subjects were required to be undergoing receptivity testing as part of their physician-directed care, for which the patients consented. All subjects were given a unique deidentifier, to which results were compared.

Figure 2: Gene Selection Process



The main objective of this non-inferiority study was to verify the efficacy of the newly developed ERPeak[SM] endometrial receptivity test to characterize the receptivity status of the endometrium. The primary outcome of this study was the assessment of concordance rates between the ERPeak[SM] test and the current clinically acceptyed test performed under clinical care.

A total of 173 endometrial tissue samples were received and tested by CooperSurgical. The green highlighted fields in Figure 4 demonstrate the concordance of results between the known test outcomes from the current clinically accepted test against the ERPeak[SM] endometrial receptivity test.

Based on these results and the assumption of absolute truth in the clinically accepted test results, the ERPeak[SM] test has an accuracy of 92.4% and an observed sensitivity and specificity of 90.0% (95%CI: 80.5– 95.9%) and 94.1% (87.5– 97.8%), respectively.

In addition to the 173 endometrial tissue samples received and tested above, CooperSurgical received and tested 8 samples for which the current clinically accepted test had classified as “Insufficient RNA”, “Invalid RNA”, or “Non-informative”. Of those samples, the CooperGenomics ERPeak[SM] test classified 5/8 samples (62.5%) as either “Pre-receptive”, “Receptive”, or “Post-receptive” and 3/8 samples (37.5%) were classified as “Non-informative”, indicating that the quality of endometrial tissue was suboptimal (Figure 5).

Figure 3: Non-inferiority Study Design

Figure 4: Sample concordance between ERPeak[SM] endometrial receptivity test and clinically accepted test

		Current clinically accepted test				
		Pre-receptive	Early-receptive	Receptive	Late-receptive	Post-receptive
ERPeak[SM] Test	Pre-receptive	56	6			
	Receptive	6	24	59	12	1
	Post-receptive					7
	Non-informative	1*		1*		

*Biopsies called non-informative by the ERPeak[SM] test were excluded from all calculations due to suboptimal tissue quality

Figure 5: Non-informative sample results breakdown

		Current clinically accepted test		
		Insufficient RNA	Invalid RNA	Non-informative
ERPeak[SM] Test	Pre-receptive	2		
	Receptive		1	1
	Post-receptive		1	
	Non-informative	2		1

Discussion and summary

The ERPeak℠ endometrial receptivity test is a genomic test that analyzes the gene expression profile of an endometrial biopsy to estimate the optimal time for embryo transfer. The validation study described here demonstrates a high degree of concordance with the clinically accepted test.

The ERPeak℠ test has an accuracy of 92.4% and an observed sensitivity and specificity of 90.0% (95%CI: 80.5–95.9%) and 94.1% (87.5–97.8%), respectively. It is important to note that even the currently accepted clinical test, used as the comparator in this study, claims a specificity of 97% and sensitivity of 90%.

Thus, in the case of the discordant samples, it is difficult to conclude which test – if either – is correct unless health outcome data is available.

Studies tracking embryo transfer time and associated obstetric outcome are ongoing and should provide valuable insight into the performance of the ERPeak℠ test. The ERPeak℠ endometrial receptivity test is capable of using this clinical data to improve through statistical learning and will benefit from regular improvements as we gather more clinical samples, testing data and outcomes.

References

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