ORIGINAL ARTICLE: GENETICS

Window of implantation transcriptomic stratification reveals different endometrial subsignatures associated with live birth and biochemical pregnancy

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Objective: To refine the endometrial window of implantation (WOI) transcriptomic signature by defining new subsignatures associated to live birth and biochemical pregnancy.
Design: Retrospective cohort study.
Setting: University-affiliated in vitro fertilization clinic and reproductive genetics laboratory.
Patient(s): Healthy fertile oocyte donors (n = 79) and patients with infertility diagnosed by Endometrial Receptivity Analysis (n = 771).
Intervention(s): None.
Main Outcome Measure(s): WOI transcriptomic signatures associated with specific reproductive outcomes.
Result(s): The retrospective cohort study was designed to perform a prediction model based on transcriptomic clusters for endometrial classification (training set, n = 529). The clinical follow-up set in the expected WOI (n = 321) was tested with the transcriptomic predictor to detect WOI variability and the pregnancy outcomes associated with these subsignatures (n = 228). The endometrial receptivity signature was redefined into four WOI transcriptomic profiles. This stratification identified an optimal endometrial receptivity (RR) signature resulting in an ongoing pregnancy rate (OPR) of 80% in terms of live birth, as well as a late receptive-stage (LR) signature with a potential high risk of 50% biochemical pregnancy. Abnormal down-regulation of the cell cycle was the main dysregulated function among the 22 genes associated with biochemical pregnancy.
Conclusion(s): The major differences between the WOI transcriptomic stratification were in the OPR and biochemical pregnancy rate. The OPR ranged from 76.9% and 80% in the late prereceptive (LPR) and RR signatures, respectively, versus 33.3% in the LR. The biochemical pregnancy rate was 7.7% and 6.6% in LPR and RR, respectively, but 50% in LR, which highlights the relevance of endometrial status in the progression of embryonic implantation. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Biochemical pregnancy signature, endometrial genomic medicine, endometrial receptivity, endometrial transcriptomic predictors, transcriptomic stratification of uterine receptivity

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Transcriptomic predictors have been applied to medicine as powerful tools for stratifying patients and subphenotype diseases, improving diagnoses and the personalization of treatments. Since the advent of the first predictors (1, 2), the guidelines and best practices have been defined, updated, and approved by the U.S. Food and Drug Administration for medical diagnosis (3). These steps ensure that transcriptomic predictors will continue to make valuable contributions to clinical treatment.

Such predictors are generated computationally by machine learning from microarray data for known samples to make predictions for unknown samples. Machine learning uses a data matrix, called a training set, as a reference. Learning can occur from a labeled training set (supervised learning, i.e., transcriptomic predictors) or by using an unlabeled microarray set to structure data and define profiles (unsupervised learning, i.e., clustering methods). Sometimes unsupervised learning can be used to supervise transcriptomic predictors, as was done for a breast cancer risk prognostic signature (1). Predictor design, especially the training set population inference and how it is supervised, is the key for good model performance. The self-assessment process, called cross-validation, is where error estimation for the model is calculated. This process consists of dividing the training data randomly into blinded and nonblinded portions and using the blinded prediction to calculate the error estimation.

Using this process to improve and introduce accurate transcriptomic predictors into reproductive medicine is crucial for disease stratification and precision medicine for complex and multifactorial fertility traits. Some preliminary transcriptomic models have been implemented in embryo aneuploidy (4) and in granulosa cells as predictive for embryo quality (5). The most extensive application of transcriptomic predictors in reproduction has been for other complex and multifactorial contributors to infertility, such as the endometrial factor (6–8). In all cases, clinical parameters have been the gold standard to supervise the models and determine the transcriptomic prediction.

Endometrial receptivity, until recently the black box of reproductive medicine, is the crucial status of the human endometrium. A receptive endometrium regulates the adhesion of the embryo, allowing pregnancy to initiate (9). Accumulated knowledge about the transcriptomic profiles related to endometrial receptivity (10, 11) led us to create the Endometrial Receptivity Analysis (ERA) (6, 7). This analysis assays the expression of 238 genes that have been demonstrated to be potential transcriptomic predictors of endometrial receptivity, enabling identification of the window of implantation (WOI)—the timing of endometrial receptivity—for each patient in a personalized manner (12–14). This first transcriptomic predictor in endometrial receptivity was built using the luteinizing hormone (LH) peak as a reference to supervise the training set (6, 7). Although this predictor was more accurate than classic endometrial histology dating and was completely consistent (7), the number of days after the luteinizing hormone (LH) peak or after progesterone administration has served as the gold standard for endometrial preparedness. We learned that different women may have varying transcriptomic profiles even if samples are taken on the same day or after the same hormone treatment regimen (12, 13).

Our work updates the prediction design supervised by transcriptomic clusters to stratify transcriptionally the WOI and to improve the training set population inference by increasing the sample size. This refinement provides more detailed insight into the use of endometrial transcriptomic predictors for patient stratification and provides a powerful methodology to describe the variation in the WOI transcriptome and the clinical meaning of these subsignatures in terms of reproductive outcome.

MATERIALS AND METHODS

This retrospective study was approved by the institutional review board of the Instituto Valenciano de Infertilidad, Valencia, Spain (1401-FIVI-002-CS).

Endometrial Sample Cohort

Initial training set. The initial training set comprised 79 healthy, regularly cycling oocyte donors aged 20–34 years with a body mass index (BMI) of 19–25 kg/m^2. Each donor's endometrial sample was timed based on the LH peak determined from the menstrual cycle of fertile women. The receptive (R) (n = 39) group was formed from samples obtained at day LH+7, and the prereceptive (PR) (n = 14) group comprised samples from days LH+1 to LH+4. The proliferative (PF) group (n = 14) included samples collected on days 8–12 of the menstrual cycle, and the postsecretory group (n = 12) consisted of samples from LH+11 to LH+13. The sample cohort was published in Díaz-Gimeno et al. (7).

New training set. The new training set comprised 450 women aged 38–43 years with a body mass index (BMI) of 19–27 kg/m^2. Each patient's endometrial sample was collected during the expected WOI, either with progesterone (P) hormone replacement therapy (P+3 to P+7) or in a natural cycle (LH+7, human chorionic gonadotropin [hCG]+7).

External validation set. Endometrial biopsy samples from infertile patients diagnosed by ERA (n = 321) were collected in the expected WOI (P+4 to P+7, LH+7, hCG+7). The receptive patients from this cohort (n = 228) underwent embryo transfer on the day indicated by transcriptomic profiling, and the pregnancy outcome was monitored.

Endometrial Sampling and Processing

Endometrial biopsy samples were collected and processed following the ERA protocol guidelines (6). Hybridized ERA microarrays with the Agilent one color protocol were scanned in an Axon 4100A, and data were extracted with the use of the Genepix Pro 6.0 software (Molecular Devices).

Microarray Preprocessing and Normalization

Gene expression values (.gpr files) were preprocessed, normalized, and statistically analyzed. Briefly, the half background median intensity values were subtracted from the average intensity of each spot and were normalized between arrays using the quantile method implemented in the Bioconductor

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