Journal of Reproductive Health and Medicine 2 (2016) 83–92

Contents lists available at ScienceDirect

Journal of Reproductive Health and Medicine

journal homepage: www.elsevier.com/locate/jrhm

Original article

Prediction of pregnancy outcome of IVF-ET cycles following endometrial injury in women with previously failed implantation based on endometrial transcriptomics: A preliminary report

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ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form 14 May 2016
Accepted 19 May 2016

Keywords:
Endometrial injury
IVF-ET
Pregnancy
Transcriptomics

ABSTRACT

Background/aims: Timed administration of endometrial scratching in natural cycles followed by IVF-ET reportedly yields improved clinical pregnancy in women. In the present study, we have examined the issue of endometrial transcriptomic response in natural cycles preceding to the intervention cycles of IVF-ET to explore the differences, if any, in transcript expression profiles between those who conceived and those who did not following endometrial scratching in IVF-ET.
Methods: Patients (n = 25) undergoing autologous IVF-ET cycles with history of failed IVF-ET/ICSI cycles underwent endometrial scratching and sampling during 12–18 day of untreated cycles preceding to intervention. Each patient was monitored for pregnancy outcome using the routine protocol. Each sample was subjected to whole genome expression array experiment (n = 20) followed by exploratory and differential display analysis.
Results: Endometrial whole genome transcriptomics of two groups (pregnancy positive, n = 10; pregnancy negative, n = 10) revealed that samples mutually clustered primarily based on pregnancy outcome. The secretory maturation of endometrium was seen to involve pathways related to oxidative phosphorylation, ubiquinone metabolism, cellular adhesion, skeletal and motor activities, and transcription and translation regulation. Additionally, genes related to neurogenesis and synaptogenesis, chemokines associated with cell adhesion, and developmental signalling were up-regulated in samples obtained from pregnancy-positive women. Further, patients with affected endometrial expression of genes involved in gynaecological neoplasm and transcriptional dysregulation in natural cycles did not conceive in IVF-ET.
Conclusion: Transcriptomic expressions in endometrium yielded information with predictive value of pregnancy outcome following endometrial scratching in women with history of repeated implantation failure upon IVF-ET.

1. Introduction

It is generally assumed that modular delineation of functional elements in maternal and embryonic compartments towards successful blastocyst implantation is the key for achieving better results and reproducibility in IVF-ET set up.[1] In contrast to the fact that embryo is capable of 'implantation' in a variety of non-endometrial tissues regardless of any specific hormonal preparation in a variety of species, embryo implantation in the uterus actually occurs only during receptive period under proper hormonal priming.[1] In 1940s, Hall anticipated that sarcoma implantation in the uterus of cyclic mice occurred at sites where the trocar used to transfer the tissue had damaged the uterine lining.[2] In 1969, Cowell reported that 4-day-old mouse embryos implanted preferably at uterine sites with denuded luminal epithelium.[3] Cowell studied nineteen implantation sites immediately after embryo transfer and observed that uterine luminal epithelia were compromised in seventeen samples, while the epithelium was intact in remaining two implantation sites.[3] Subsequent experiments have clearly revealed that intra-endometrial embryo transfer can lead to successful implantation bypassing stringent uterine receptivity.[4–6] However, the auxiliary fact that

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http://dx.doi.org/10.1016/j.jrhm.2016.05.003
2214-420X/

luminal injury may 'somehow' support at least a subset of embryos to implantation even at the intact sites had escaped any meaningful attention despite the Karow report in 1971, only two years after Cowell's seminal paper. Karow et al. reported that only two of twenty-eight women aborted, who conceived in the same cycles that underwent endometrial biopsy in the luteal phase.[7] The idea that timed endometrial injury may improve embryo implantation came back with higher acceptance relatively recently following Barash report in 2003.[8] In the Barash study, 45 women who failed to conceive after one or more cycles of IVF-ET and who were subjected to endometrial injury in the cycle before IVF showed significantly improved pregnancy outcome. In their study, the endometrial injury was performed on days 8, 12, 21, and 26 of the cycle preceding IVF. The rates of implantation, clinical pregnancy, and live birth in the endometrial injury group were 28%, 67%, and 49% and in the control group were 14%, 30%, and 23%, respectively.[8] This was followed by a large number of similar trials based on which it has been concluded that endometrial injury performed in preceding cycle to the embryo transfer cycle improved clinical pregnancy and live birth rates in women undergoing ART.[9–14] However, the physiological basis of improved pregnancy outcome following endometrial injury is, as yet, a matter of conjecture only.[15,16] It is plausible that the issue of endometrial competence in terms of its expression in response to endometrial injury plays a critical role in mediating pregnancy outcome upon IVF-ET.[1,17] To this effect, in the present study, we have examined the genome-wide transcript expressions in endometrial tissue samples collected from the natural cycles preceding to the intervention cycles of IVF-ET in order to record differences in transcript expression profiles, if any, between those who conceived and those who did not. It was anticipated that exploratory and differential analysis of genome-wide expression data may yield interesting leads to identify markers of endometrial origin for potentially successful ART outcome.

2. Materials and methods

2.1. Subjects

This was a prospective, interventional study which was approved by the Institutional Ethics Committee and it was conducted between November 2012 and May 2014 involving patients undergoing IVF treatment at the Department of Obstetrics and Gynaecology in All India Institute of Medical Sciences (AIIMS), New Delhi with compliance to all the ethical norms and practices. The patients undergoing fresh autologous IVF-ET cycles and with documented evidence of at least two previously failed IVF-ET/ICSI cycles and that of being good responders in the previous IVF cycle were recruited in the present study. Patients who had developed at least four good-quality embryos (grades 1 and 2 according to Veeck's grading) in the previous IVF cycles were taken as 'good responder'.[18] Patients detected to have endometrial tuberculosis, intramural fibroid, submucosal myoma, and Asherman's syndrome, and hydrosalpinx, as well as, with age more than 36 years were excluded from the study. Patients ($n = 25$) who were found suitable for the study were explained about the study objectives and design and offered to undergo endometrial scratching on days 12–18 prior to the ovarian stimulation cycle. Informed consents were obtained from those who were willing to participate in this interventional and discovery-oriented study. Thus, the patients, clinicians and scientists were aware of the study design.

2.2. Patient monitoring and sample collection

All patients were evaluated with baseline data of FSH, AMH, antral follicle count on day 3, and they underwent endometrial scratching and sampling during 12–18 day of untreated cycles preceding to intervention cycles. For endometrial injury and sampling, the Pipelle was introduced into the uterine cavity, it was rotated 360° and moved up and down three times after withdrawing the piston.[14] The tissue samples were immediately brought to the Molecular Biology Laboratory in the Department of Physiology, AIIMS, New Delhi for further processing and transcriptomic study as detailed in the following section.

2.3. Intervention cycle

Each woman recruited in the study underwent either GnRH agonist or GnRH antagonist protocols as described elsewhere.[19] Women were scheduled for oocyte retrieval when at least three follicles reached a size of 18 mm. Oocyte retrieval was performed by the transvaginal route under ultrasound guidance, 34–36 h after rhHCG trigger with 250 μg s.c. The morphology of each aspirated oocyte was noted after denudation with hyaluronidase. Conventional IVF and ICSI was performed on patients, the details of which are given in Table 1. Up to three grade 1 or 2 embryos as per Veeck's grading scale were transferred with a Wallace® catheter (Smith Medical International Ltd., Hythe, Kent, UK) on day 5 under ultrasound guidance. Luteal phase was supported with 600 mg/day of micronized progesterone vaginally and 4 mg of estradiol valinate orally till 12 weeks of pregnancy.

2.4. Outcome measures

A serum β-hCG measure >50 μIU/ml during 12–14 days after the embryo transfer was accepted as biochemically positive pregnancy. Women with positive biochemical pregnancy test results were further assessed by ultrasonography for the number of gestational sacs and the presence of cardiac activity 2 weeks later. A positive cardiac activity was defined as clinical pregnancy and pregnancies reaching to gestational week 12 were considered as ongoing pregnancy. The primary outcome measure was live birth rate, the secondary outcome measures were clinical pregnancy and implantation rates.

2.5. Experimental procedure

The methodological details of RNA extraction followed by the estimation of its yield and purity using standard electrophoretic and spectrophometric protocols and its RIN score using the Agilent 2100 Bioanalyzer, RNA 6000 Nano LabChip kit and Agilent 2100 Expert Software (Agilent Technologies, Santa Clara, CA, USA) have been given elsewhere.[20,21] Individual RNA samples from tissue samples ($n = 20$) from confirmed cases and having RIN scores >8.0 were subjected to whole transcriptome array experiment using the Agilent Whole Human Genome 60-mer 4X44K microarray according to the manufacturer's recommendations. Table 1 gives the details of the samples used in the present study. Hybridized arrays were scanned with Agilent's G2505B microarray scanner system and the raw data were imported into GeneSpring 13.1.1 software (Agilent Technologies, Santa Clara, CA, USA) for further analysis. Pearson's correlation coefficients done to assess the reliability of data obtained from two separate hybridization runs for same RNA preparation for four (4) samples confirmed the reproducibility assurance ($P < 0.01$) among hybridizations from separate chips with the same RNA samples as they yielded QC statistics highly concordant with that of the manufacturer, and it revealed more than 95% confidence level.