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# Endometrial transcriptome analysis indicates superiority of natural over artificial cycles in recurrent implantation failure patients undergoing frozen embryo transfer

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**Abstract** Little consensus has been reached on the best protocol for endometrial preparation for frozen embryo transfer (FET). It is not known how, and to what extent, hormone supplementation in artificial cycles influences endometrial preparation for embryo implantation at a molecular level, especially in patients who have experienced recurrent implantation failure. Transcriptome analysis of 15 endometrial biopsy samples at the time of embryo implantation was used to compare two different endometrial preparation protocols, natural versus artificial cycles, for FET in women who have experienced recurrent implantation failure compared with fertile women. IPA and DAVID were used for functional analyses of differentially expressed genes. The TRANSFAC database was used to identify oestrogen and progesterone response elements upstream of differentially expressed genes. Cluster analysis

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demonstrated that natural cycles are associated with a better endometrial receptivity transcriptome than artificial cycles. Artificial cycles seemed to have a stronger negative effect on expression of genes and pathways crucial for endometrial receptivity, including *ESR2*, *FSHR*, *LEP*, and several interleukins and matrix metalloproteinases. Significant overrepresentation of oestrogen response elements among the genes with deteriorated expression in artificial cycles ($P < 0.001$) was found; progesterone response elements predominated in genes with amended expression with artificial cycles ($P = 0.0052$).



**KEYWORDS:** artificial cycle, endometrial receptivity, frozen embryo transfer, hormone response elements, recurrent implantation failure, unexplained female infertility

## Introduction

The current trend towards cryopreservation of all embryos after IVF, with transfer of a thawed embryo in a subsequent cycle now contributes more substantially than ever to the cumulative live birth rates after IVF treatment. Together with the evidence of improved endometrial receptivity in cycles without ovarian stimulation (Roque et al., 2013; Shapiro et al., 2011, 2013), the increased success rate of frozen embryo transfer (FET) now equals or even betters that of IVF with fresh embryo transfer (Allersma et al., 2013; Roque et al., 2015; Shapiro et al., 2014; Wong et al., 2014). Furthermore, the better perinatal outcomes of preterm birth, low birth weight and being small for gestational age among singletons born after the transfer of frozen–thawed embryos compared with infants born after ovarian stimulation and IVF (Ishihara et al., 2014; Maheshwari et al., 2012; Pinborg et al., 2013; Wennerholm et al., 2013) have led to an important shift from fresh embryo transfers in IVF towards a freeze-all strategy (Evans et al., 2014; Wong et al., 2014).

A crucial aspect of FET cycles is the timing and preparation of the endometrium to receive the transferred embryo(s). Protocols used in FET include natural cycle (NC-FET) and artificial cycle (AC-FET) with or without preceding pituitary down-regulation through GnRH agonist co-treatment (Hill et al., 2010). Patients undergoing AC-FET start with daily oestrogens, supplemented with progesterone when the endometrium has reached sufficient thickness (Groenewoud et al., 2012). Although the advantages of natural cycles FET (NC-FET) such as less medication and cheaper price somewhat counterbalance the need for more frequent ultrasonographic evaluation of the dominant follicle, the risk of unexpected ovulation and insufficient endometrial development in these cycles (Groenewoud et al., 2012), the clinical preference for the predictability and reliability of AC-FET has prevailed (Givens et al., 2009). So far, however, no clear data support one endometrial preparation method over another. In a recent systematic review and meta-analysis, it was concluded that there are no differences in the clinical pregnancy rate, ongoing pregnancy rate or live birth rate in connection with the different methods of endometrial preparation before FET (Groenewoud et al., 2013). Furthermore, despite active investigation of the endometrial transcriptome that clearly demonstrates an unfavourable endometrial gene expression profile during hormonal stimulation in ovarian stimulation (Haouzi et al., 2009a; Horcajadas et al., 2008; Ruiz-Alonso et al., 2012), there is no knowledge of how and to what extent hormonally supplemented cycles influence endometrial preparation for embryo implantation at a molecular level.

In the present study, endometrial gene expression profiles in infertile women undergoing two different endometrial preparation protocols (AC-FET and NC-FET) was compared with fertile women in a natural cycle. Our study group of women who had experienced recurrent implantation failure (RIF), also diagnosed as unexplained infertility, is especially intriguing as we have demonstrated altered endometrial receptivity in these women (Aghajanova et al., 2009; Altmäe et al., 2010). Therefore, we aimed to clarify whether AC-FET with oestrogen and progesterone improves endometrial maturation in our study group. To answer this question, we aimed to identify two groups of genes with opposite transcriptional behaviour in patients who have experienced RIF. First, we were interested in the genes, which show abnormal expression in the natural cycle, but are amended in artificial cycles and, second, the opposite case of genes showing normal expression in natural cycles, with deteriorated gene-activity after administration of steroid hormones in artificial cycles. By analysing the genes in both categories, the aim was to arrive at conclusions about the pros and cons of using artificial cycles in FET.

Endometrial tissue is one of a few tissues that are overwhelmingly controlled by steroid hormones, oestradiol and progesterone. These steroid hormones act as transcriptional regulators via ligand-bound receptor complexes interacting with the DNA consensus sequences in target genes, referred to as hormone response elements (HRE). Although major progress has been made in deciphering the HRE for oestrogen response elements and progesterone response elements, little is known about their roles in embryo implantation. Additionally we set out to analyse the oestrogen response elements and progesterone response element sequences –50 kb upstream of genes that were differentially expressed after artificial cycles or related to infertility.

The aforementioned aims are critical in obtaining a better understanding of the mechanisms of steroid hormone involvement in endometrial maturation, and in the long term this knowledge should help to devise better hormonal regimens for FET, even for patients with the complication of RIF.

## Materials and methods

### Study design and endometrial biopsy sample collection

In total fifteen endometrial biopsy samples were obtained from women with unexplained RIF in NC-FET ($n = 5$), women with unexplained RIF in AC-FET ($n = 5$), and from healthy women with proven fertility in natural cycles (NC-FC) ($n = 5$). The