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ARTICLE

Is endometrial receptivity transcriptomics affected in women with endometriosis? A pilot study

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Abstract Endometrial receptivity is still questioned today in women with endometriosis. The aim of this study was to assess the endometrial receptivity gene signature in patients with different stages of endometriosis by investigating transcriptomic modifications of their endometrium using the endometrial receptivity array (ERA) test. A prospective, interventional multicentre pilot trial was designed and implemented in two university-affiliated infertility units from Belgium and Spain. Gene expression microarray was used to diagnose the receptivity status by quantifying the expression of 238 specific genes directly related to human endometrial receptivity. Unsupervised hierarchical clustering showed no clustering of samples based on endometriosis stages. Two subgroups of samples clustered together corresponding on the day of the cycle in which the biopsy was taken (day 18 versus days 19–20). None of the 238 genes present in the ERA array were significantly over- or under- expressed in any of different stages of the disease compared with controls. Minimal differences were found when looking at the functional profile, suggesting that the possible effect from a clinical point of view may be meaningless. Endometrial receptivity gene signature during the implantation window does not vary significantly among patients with endometriosis even considering different stages compared with healthy women.

KEYWORDS: endometrial receptivity, endometriosis, gene array

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Introduction

The general 'dogma' that endometriosis is associated with infertility still lacks consensus about consistent functional mechanisms by which this disorder may diminish cycle fecundity (Giudice, 2010). Folliculogenesis, ovulation, ovum transport, sperm quality and transport, fertilization, embryo quality and luteal function may or may not be altered in women with such a pathological condition (de Ziegler et al., 2010).

Accumulated evidence suggests that endometriosis in natural and IVF cycles affects human embryo implantation (Arici et al., 1996; Barnhart et al., 2002; Jacobson et al., 2010). Our group has consistently demonstrated at the clinical level that endometriosis is not detrimental to embryo implantation in ovum recipients (Budak et al., 2007; Diaz et al., 2000; Simon et al., 1994). Endometrial markers, however, have been reported to be significantly different in eutopic endometrium between women with endometriosis compared with women without endometriosis (May et al., 2011), and further association between altered expression of several markers and impaired embryo implantation of endometrial origin has been proposed in women with endometriosis (Daftary et al., 2002; Lessey et al., 1994; Revel, 2012; Taylor et al., 1999; Wei et al., 2009). Candidate endometrial markers that have been reported to be disrupted are αvβ3 integrins, methylation of HoxA10 — a known stimulator of αvβ3 expression — glycodelin A, osteopontin, lysophosphatidic acid receptor, hepatocyte growth factor, 17-β-hydroxysteroid dehydrogenase, leukaemia inhibitory factor, matrix metalloproteinases, endometrial bleeding factor or indian hedgehog (Daftary et al., 2002; Lessey et al., 1994; Revel, 2012; Taylor et al., 1999; Wei et al., 2009). Steroid hormone pathways may also be altered in women with endometriosis. In fact, an up-regulation of oestrogen receptors as well as progesterone resistance status owing to the absence of the beta isoform of its receptor has been described (Lessey et al., 1988; Wu et al., 2006).

As technology has evolved, the single molecule approach has now become obsolete. We are now in the position to investigate complex functions, such as endometrial receptivity, using a more integrated approach by evaluating its transcriptomic signature (Aghajanova et al., 2008). Several investigators have reported differential gene expression in the eutopic endometrium from women with endometriosis compared with controls using mRNA microarray analysis (Absenger et al., 2004; Burney et al., 2007; Kao et al., 2003; Sherwin et al., 2008), specifically during early (Burney et al., 2007), mid-luteal (Kao et al., 2003) and late luteal phase (Sherwin et al., 2008). Other groups have analysed human endometrial receptivity genes but unrelated to endometriosis, and has therefore not been discussed.

The endometrial receptivity array (ERA) is a validated diagnostic assay that evaluates the expression of 238 selected genes that play a crucial role for the development of endometrial receptivity during the window of implantation (Diaz-Gimeno et al., 2011). The accuracy and consistency of the ERA diagnostic tool has been demonstrated to be superior to endometrial histology, and results are completely reproducible 29–40 months after the first ERA test (Diaz-Gimeno et al., 2013). Its diagnostic value guiding personalized embryo transfer has been recently demonstrated in patients with repeated implantation failure (Ruiz-Alonso et al., 2013).

The present study aimed to assess the endometrial receptivity gene signature in patients with different stages of endometriosis by investigating transcriptomic modifications of their endometrium using the ERA test.

Material and methods

Patients and samples

Endometrial samples, collected during laparoscopic surgery from infertile women with endometriosis ($n = 17$) and infertile women without endometriosis ($n = 5$), were selected from the Biobank of the Endometriosis and Reproductive Medicine Research Unit at the University Hospitals of Leuven, Belgium (**Table 1**). The stage of endometriosis was based on the revised staging system of the American Society for Reproductive Medicine (ASRM, 1997). In control patients, the absence of endometriosis was laparoscopically confirmed. In both groups, any other pelvic abnormality, such as fibroids or adenomyosis, were not present. All samples stored in this biobank had been obtained from women who had provided written informed consent using forms that had been approved by the Commission for Medical Ethics from the Faculty of Medicine (Leuven University, KU Leuven). This study was approved by the Clinical Trial Commission and by the Biobank Board of Leuven University Hospitals on 3 September 2012 (reference number S54508). Most of the samples ($n = 18$) were previously used in another study (Fassbender et al., 2012), whereas a further four endometrium RNA samples from women with endometriosis were used for this study only. Endometrial samples were blindly sent from Leuven to IVIOMICS where the analysis was carried out.

The following inclusion criteria were used for the selection of these endometrial samples: absence of hormonal treatment during the last 3 months before laparoscopic surgery;

Table 1 Epidemiologic characteristics of the study population.

	Endometriosis (n = 17)	*Control (n = 5)*
Age (years, mean ± SD)	31.3 ± 2.3	33.2 ± 1.3
Stages		
Minimum (I)	I = 7	
Mild (II)	II = 3	
Moderate (III)	III = 4	
Severe (IV)	IV = 3	
Infertility		
Primary	P = 12	P = 1
Secondary	S = 4	S = 4
Pain		
Chronic pelvic pain	CPP = 3	CPP = 0
Dyschezia	Dh = 1	Dh = 0
Dysmenorrhoea	Dm = 9	Dm = 2
Dyspareunia	Dp = 2	Dp = 1
Cycle phase	Luteal	Luteal
Day of cycle	18–20	18–20

CPP = chronic pelvic pain; Dh = dyschezia; Dm = dysmenorrhoea; DP = dyspareunia; P = primary; S = secondary.