High endometrial aromatase P450 mRNA expression is associated with poor IVF outcome

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BACKGROUND: The success of IVF treatment is dependent upon embryo quality and coordinated growth and differentiation of the endometrium. Aromatase P450 expression in the human endometrium is thought to be restricted to women with proliferative reproductive tract disorders such as endometriosis, leiomyomas and adenomyosis. METHODS: To determine whether endometrial aromatase P450 mRNA expression is prognostic of IVF outcome, we quantified transcript levels in biopsy specimens from a cohort of subfertile patients awaiting IVF treatment using real-time quantitative PCR. RESULTS: Aromatase P450 transcripts were detected in all endometria examined, although the levels varied considerably between samples, ranging from 0.22 to 486.6 arbitrary units (a.u.). The clinical pregnancy rate in women with high endometrial aromatase P450 mRNA levels (>8.3 a.u.; n = 21) was 9.5% compared with 30.1% in those patients with low expression levels (<8.3 a.u.; n = 101) (P < 0.05). The cycle day of the endometrial biopsy, cause of infertility, age, parity, number of oocytes collected and number of embryos transferred did not differ between patients with high versus low endometrial aromatase P450 mRNA levels (P > 0.1). CONCLUSIONS: Our results indicate that endometrial P450 mRNA levels can identify women at increased risk of IVF treatment failure.

Key words: aromatase/endometrium/IVF/mRNA/pregnancy outcome

Introduction

Twenty-five years on from the birth of Louise Brown, over 250 000 IVF treatment cycles are recorded yearly on the European IVF monitoring programme (Nygren et al., 2002). Despite the phenomenal growth in infertility services worldwide, the live birth rates after IVF have changed little over the last decade, and remain ~20–25% per treatment cycle started (Nygren et al., 2002). Two major obstacles are thought to be responsible for the low implantation rate and high early pregnancy wastage after IVF. First, there is a high incidence of aneuploidies in human oocytes and preimplantation embryos, and considerable efforts are being made to improve embryo selection and to screen for chromosomal defects prior to embryo transfer (Hardy et al., 2002; Gianaroli et al., 2003). A second limitation is that there are no clinically useful markers to determine whether the host endometrium is adequately prepared to facilitate adhesion, implantation and development of the transferred embryo(s). Attempts have been made to determine whether markers of the limited period of endometrial receptivity (implantation window), such as endometrial cytokine production and α,β3 integrin expression on surface epithelium, are prognostic of subsequent pregnancy (Lindhard et al., 2002; Ordi et al., 2003). However, this approach is clinically cumbersome as it depends on timed biopsy samples, and the value of these markers in predicting IVF outcome remains unproved (Lindhard et al., 2002).

The CYP19 gene encodes aromatase P450, a cytochrome P450 enzyme that catalyses three consecutive hydroxylation reactions converting C19 androgens to C18 estrogenic steroids (Sebastian et al., 2001; Simpson et al., 2002; Chen et al., 2002). Previous reports indicated that aromatase P450 mRNA is expressed in the endometrium of women with benign and malignant reproductive tract disorders, but not in endometria of disease-free controls (Noble et al., 1996; Kitawaki et al., 1997; 1999; Dheenadayalu et al., 2002). We postulated that the abundance of aromatase P450 transcripts in the endometrium could reflect the degree of biochemical impairment and serve as a prognostic marker of IVF outcome. To test this hypothesis,
the relative aromatase P450 transcript levels were determined in endometrial biopsies, taken randomly in the cycle, from 150 women prior to starting IVF treatment.

**Materials and methods**

Patients were recruited from eight European IVF centres, written consent was obtained, and the study received approval from the local research ethics committee of each participating centre. All women had regular menstrual cycles and were not on hormonal treatment at the time of biopsy. Endometrial samples were taken 1–4 months before IVF treatment. Biopsy samples were immediately immersed in RNA later (Ambion) and stored according to the manufacturer’s recommendations until transportation to the central laboratory. Total RNA was isolated from tissues using STAT-60 (Tel-Test) and treated with DNaseI. Equal amounts of total RNA (2 μg) were reverse transcribed using the Superscript First-Strand Synthesis System for RT±PCR (Invitrogen) and the resulting first-strand cDNA was diluted and used as template in the real-time quantitative (RTQ)-PCR analysis. Detection of aromatase P450 and GAPDH expression was performed with an ABI PRISM 7700 Sequence Detection System (Applied Biosystems), using the relative standard curve method. GAPDH represents a non-regulated gene, and its expression served as internal control and was used to normalize for variances in input cDNA. All measurements were performed in triplicate. The following gene-specific primer pairs and probes were designed using the ABI Primer Express software: GAPDH forward primer (GAAGGTGAAGGTCGGAGT), GAPDH reverse primer (GAAGATGGTGATGGGATTTC), GAPDH probe (5¢ 6FAM, 3¢ TAMRA) (ATTTGGTCGTATTGGGCGCCTGGTCACC), aromatase P450 forward primer (GGCATACCTCCTATGGGTTGTC), aromatase P450 reverse primer (GTAGCCTGGTTCTCTGTGTGAA) and aromatase P450 probe 3 (5¢ 6FAM, 3¢ TAMRA) (CCAAAGCTAGGTGCTATTGGTCATCTGCTCCT).

Statistical analysis was performed using Student’s t-test for parametric variables and the χ²-test for categorical variables. A P-value <0.05 was considered statistically significant. The sample size was calculated on the assumption that 50% of the IVF/ICSI patients would express endometrial aromatase, and that in this group the pregnancy rate per transfer is reduced from 35% to (a) 10% or (b) 15%. With the level of significance of 0.05 and with 80% power, the number needed for (a) is 51 and for (b) 83.

**Results**

Of the 150 patients that started IVF treatment, 17 had no embryo transfer due to failed superovulation (n = 5), failed oocyte collection (n = 5) or non-fertilization (n = 7). The biopsy sample was deemed inadequate for analysis in 11 of 133 (8.2%) patients who had a successful embryo transfer. Aromatase P450 transcripts were detected in all remaining 122 informative cases, although the levels varied considerably between samples, ranging from 0.22 to 486.6 arbitrary units (a.u.) (>2200-fold difference). The overall clinical pregnancy rate, defined as the proportion of women with ultrasound evidence of a viable intrauterine pregnancy, in our study population was 27%. The mean aromatase P450 mRNA levels in women who subsequently became pregnant following IVF treatment was 14.6 a.u. (SD 54), compared with 15.2 a.u. (SD 68.6) in patients with IVF failure (P > 0.05; Figure 1). Aromatase P450 transcripts were also measured in the 17 samples obtained from patients who subsequently had no embryo transfer. The mean aromatase P450 mRNA expression level in this group was 12.6 a.u. (SD 22.9), which was not significantly different from the mean expression levels in patients who were successful in their treatment cycle (P > 0.05).

However, the clinical pregnancy rate per embryo transfer in patients with high endometrial aromatase P450 mRNA levels (defined as ≥8.3 a.u.; n = 21) was 9.5%, compared with 30.1% in those patients with low expression levels (<8.3 a.u.; n = 101).
(P < 0.05). This cut-off value was chosen because it best discriminated between successful and failed IVF cycles. Moreover, IVF was successful in eight of 19 (42%) women with very low transcript levels (<1.0 a.u.). Cause of infertility, age, parity, number of oocytes collected and number of embryos transferred did not differ significantly between patients with high versus low endometrial aromatase P450 mRNA levels (Table I). The level of endometrial aromatase P450 transcripts detected did not correlate with the day of the menstrual cycle at the time of biopsy (Figure 2). This was confirmed by subanalysis of transcript levels in endometrial samples from the 33 women who subsequently became pregnant after IVF and, hence, were unlikely to have had significant endometrial pathology at the time of biopsy. One patient with an expression level >300 a.u. was excluded from this analysis. The mean mRNA expression levels before day 14 of the cycle was 3.4 a.u. (SD 2.6; n = 17), compared with 3.3 a.u. (SD 4.7; n = 15) on day 14 or later (P > 0.5). These results further demonstrate a lack of correlation between aromatase P450 mRNA expression in total endometrial biopsies and the phase of the cycle.

Discussion

Aromatase P450, the estrogen synthase that converts androgen to estrogen, is physiologically expressed in a variety of tissues, including the ovary, placenta, skin, adipose tissue and brain (Hinshelwood et al., 1997; Sebastian et al., 2002; Simpson et al., 2002). In certain pathological conditions, such as breast cancer and pelvic endometriosis, very high expression levels have been reported (Noble et al., 1996; Zeitoun and Bulun, 1999; Zeitoun et al., 1999; Chen et al., 2002; Simpson and Dowsett, 2002). In the endometrium, aromatase P450 is also expressed under pathological conditions, and local estrogen biosynthesis is thought to be integral to the pathophysiology of a variety of uterine disorders, including adenomyosis, fibroids and endometriosis (Kitawaki et al., 1999; Dheenadayalu et al., 2002; Ishihara et al., 2003). Regulation of human aromatase
expression is complex in that at least nine different tissue-specific promoter regions have been identified so far (Sebastian and Bulun, 2001; Sebastian et al., 2002). Recent studies have suggested that aberrant expression of aromatase P450 in endometrium and endometriosis is effected through an imbalance between diverse transcriptional activators (e.g. SF-1) and repressors (e.g. COUP-TF, DAX-1 and WT1) (Zeitoun and Bulun, 1999; Zeitoun et al., 1999; Gurates et al., 2002).

We hypothesized that the level of aromatase P450 mRNA expression could reflect the degree of biochemical perturbation in the endometrium. To test this, endometrial aromatase P450 transcripts were quantified by RTQ-PCR in a cohort of infertile patients and correlated with subsequent IVF outcome. Twenty-one of 122 (17%) patients had elevated transcript levels (>8.3 a.u), and although IVF outcome was indeed very poor, two successful pregnancies did occur in this group. Our study also demonstrates that aromatase P450 transcripts are detectable in all biopsy samples and throughout the cycle, albeit at relative low level in the majority of cases. Moreover, the cause of infertility did not differ significantly between patients with high versus low endometrial aromatase P450 mRNA levels, but this is not entirely surprising as it is known that categorization of infertility did not differ significantly between patients with fibroids and endometriosis, suggesting that the type of ovarian down-regulation and stimulation protocol used may have a profound impact on subsequent pregnancy rates in affected patients.

This study shows that the endometrium can be an unique source of potential markers that could be exploited clinically to tailor infertility treatment to individual patients. This notion is further supported by recent microarray studies demonstrating an astonishing diversity of endometrial genes that are aberrantly expressed during the implantation window in women with endometriosis compared with healthy controls (Kao et al., 2002; 2003). For clinical translation, however, timing of the biopsy sample should ideally not matter, and it appears very likely that powerful screening tools, such as microarrays and proteomics, will uncover additional genes that are aberrantly expressed throughout the cycle. Finally, the observation that a subgroup of infertile women express high levels of aromatase P450 in the endometrium also provides a rational basis to test the use of highly selective aromatase inhibitors, such as letrozole and anastrozole, in the treatment of infertility (de Ziegler, 2003; Mitwally and Casper, 2003). However, whether expression at mRNA level correlates with protein level or aromatase P450 enzymatic activity remains to be determined.

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References


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