Endometrial secretion aspiration prior to embryo transfer does not reduce implantation rates

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Abstract

Analysis of protein patterns in endometrial secretion fluid may offer a relatively non-invasive means of assessing endometrial receptivity during fertility treatment cycles. In order to study the impact of the removal of endometrial secretions on embryo implantation, a prospective matched controlled study was performed. In 66 women undergoing IVF, endometrial fluid was obtained transcervically by aspiration just prior to embryo transfer (study group). Biochemical and ongoing pregnancy rates were compared with 66 control patients matched for stimulation treatment protocol, age, number of collected oocytes and number of high quality embryos. The protein content and uterine fluid protein profile in each sample was determined. Respective biochemical and ongoing pregnancy rates per embryo transfer were 36 and 33% in patients who underwent aspiration of endometrial secretion, compared with 33 and 30% respectively in matched control patients (P = 0.84 and P = 0.85). The protein content in endometrial fluid was sufficient for protein pattern analysis. Uterine fluid aspiration prior to IVF embryo transfer is a safe method for obtaining sufficient material for uterine secretion electrophoresis, thus allowing analysis of protein patterns serving as receptivity markers during treatment cycles. This technique may offer a novel tool for assessing endometrial receptivity during treatment cycles without affecting implantation rates.

Keywords: aspiration, endometrial receptivity, endometrium, IVF, pregnancy, secretion

Introduction

Successful implantation depends on a complex process of interaction between the embryo and the endometrium. Only if the endometrium is receptive to the embryo will apposition, implantation and trophoblast invasion occur. The period for which the endometrium is receptive to embryo implantation appears to be limited and is often referred to as the ‘window of implantation’. This widely used term emerged following investigations of the role of oestrogens in the control of the receptivity of endometrium to apposition and implantation of the blastocyst (Psychoyos, 1963; McLaren, 1973; Finn and Martin, 1974). The terms ‘receptivity’ and ‘implantation window’ are currently used synonymously to describe the physiological and structural stage of the endometrium during the luteal phase of the cycle in which attachment and implantation can be achieved. The time frame of the implantation window has been defined differently by various investigators according to different cell biological markers and levels of methodological resolution (Beier-Hellwig et al., 1989; Bergh and Navot, 1992; Lessey et al., 1995; Nikas et al., 1995).

For more than 50 years, the secretory transformation of the endometrium has been assessed by histological dating according to the criteria of Noyes (Noyes et al., 1950). More recently, immunohistochemical molecular markers (including cytokines and adhesion molecules) have been used for cell biological assessment of receptivity (for review, see Giudice, 1999). For such studies, endometrial tissue should ideally be
The analysis of endometrial secretion may constitute an alternative, less invasive technique. The ‘cross-talk’ that occurs between the embryo and endometrium prior to and during the process of implantation results in production and release of molecules into endometrial secretion. The expression of these molecules is temporally related to the phase of endometrial development (Lessey et al., 2000). Previous studies have reported detectable expression of leukaemia inhibitory factor (LIF) (Cullinan et al., 1996; Laird et al., 1997; Ledee-Bataille et al., 2002), glycodelin (PP14) (Li et al., 1993a), interleukins (Simón et al., 1998), macrophage colony-stimulating factor (M-CSF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) (Classen-Linke et al., 2000), insulin-like growth factor binding protein-1 (IGFBP-1), prolactin and human chorionic gonadotrophin (HCG) (Licht et al., 1998) in the endometrial secreted fluid, obtained during the luteal phase. In recent years, an approach has been described whereby endometrial secretion directly aspirated from uterine cavity can be analysed for the expression of these and additional proteins by electrophoretic techniques (Beier-Hellwig et al., 1998). It has been shown that the protein profile expressed in endometrial secretions alters with the phase of the cycle, and that characteristic patterns are predictive of receptivity, and subsequent implantation and pregnancy (Beier and Beier-Hellwig, 1998; Beier, 2000).

The aim of this study was to assess whether aspiration of endometrial secretions prior to embryo transfer for analysis of receptivity in a treatment cycle might be carried out without disrupting the process of implantation.

### Materials and methods

#### Subjects and treatment

The study was approved by the local ethics committee of the Erasmus Medical Centre. After informed consent, 66 women undergoing IVF were recruited to the study regardless of the individual indications for IVF. The patients were treated with recombinant FSH (Puregon; Organon Nederland BV, Oss, The Netherlands) and gonadotrophin-releasing hormone (GnRH) agonist (triptorelin; Ferring BV, Hoofddorp, The Netherlands) or GnRH antagonist (cetrorelix; Serono Benelux BV, The Hague, The Netherlands). Oocytes were retrieved and fertilized in vitro. After 3–5 days of culture, no more than two embryos, which were selected according to a previously described classification (Huisman et al., 2000), were transferred. Immediately prior to embryo transfer, aspiration of the endometrial secretion was performed as described below. Discomfort and side effects were assessed. A pregnancy test was performed 18 days after oocyte retrieval, and if positive, an ultrasound was performed 5 weeks later to assess the presence of a gestational sac and fetal heart activity.

The control patients were matched for stimulation treatment protocol, age, number of oocytes retrieved and number and quality of embryos available for transfer. They underwent embryo transfer over the same period as the study patients, and all embryo transfers and endometrial secretion aspirations were performed by the same investigator (MvdG).

#### Aspiration procedure

In all 66 patients who underwent transcervical uterine aspiration, endometrial secretion was obtained. Two catheter systems were tested. Initially, an embryo transfer catheter (Repromed®; International Medical Products BV, Zutphen, The Netherlands) with only one outlet was tested. In order to increase the volume of secretion available for analysis, a double outlet soft insemination catheter (ASSA med GmbH, Bexbach, Germany), stiffened with the stylette of the guide catheter of the embryo transfer set, was substituted after 20 patients.

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### Table 1. Patient characteristics and outcome.

<table>
<thead>
<tr>
<th></th>
<th>Study group (n = 66)</th>
<th>Control group (n = 66)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)a</td>
<td>34 ± 3.9</td>
<td>34 ± 3.7</td>
<td>0.86c</td>
</tr>
<tr>
<td>No. retrieved oocytesa</td>
<td>9.1 ± 5.4</td>
<td>9.1 ± 4.8</td>
<td>0.96a</td>
</tr>
<tr>
<td>No. obtained embryosb</td>
<td>5.2 ± 3.6</td>
<td>5.1 ± 3.3</td>
<td>0.84d</td>
</tr>
<tr>
<td>Embryo scoreb</td>
<td>2 (1–4)</td>
<td>2 (1–4)</td>
<td>0.59d</td>
</tr>
<tr>
<td>No. of embryo transfers 3/4/5 days after oocyte retrievalb</td>
<td>30/16/20</td>
<td>30/16/20</td>
<td>–</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>29/125 (23)</td>
<td>23/127 (18)</td>
<td>0.32c</td>
</tr>
<tr>
<td>Positive pregnancy test (%)</td>
<td>24 (36)</td>
<td>22 (33)</td>
<td>0.84d</td>
</tr>
<tr>
<td>Ongoing pregnancy (%)</td>
<td>22 (33)</td>
<td>20 (30)</td>
<td>0.85f</td>
</tr>
</tbody>
</table>

*aMean ± SD; bMedian (range); cStudent’s paired t-test; dWilcoxon matched pairs signed rank sum test.

*χ² test; fMcNemar test.
With the patient lying in the lithotomy position, the cervix was cleansed with a swab after insertion of a speculum. The catheter was gently introduced transcervically into the uterine cavity. When the catheter was correctly positioned, the stylette was removed and a 10 ml syringe was connected to the insemination catheter. Suction was gradually applied. Following the application of suction, the catheter was clamped just distal to the external os of the cervix with an artery clamp, and removed. The tip was cut off and placed in an Eppendorf cup with 1 ml of distilled water and frozen to –20°C.

Immediately thereafter, the normal embryo transfer procedure was carried out.

Statistical analysis

In order to exclude a major reduction in implantation as a result of fluid aspiration, a power calculation was carried out which indicated that at least 60 subjects were required in each arm to demonstrate a reduction in pregnancy rates per embryo transfer from 30 to 10% with a $P < 0.05$ and 80% power.

Possible differences between the study and control group in age, number of collected oocytes, number and quality of obtained embryos, and day of the embryo transfer after oocyte retrieval were analysed by Student’s paired $t$-test or the Wilcoxon matched pairs signed rank sum test, after the study patients were matched with the control group. For analysis of the impact of aspiration on outcome, data from both catheter systems were combined. Implantation rate differences were tested by the $\chi^2$ test. Differences in positive pregnancy test rate and the ongoing pregnancy rate were analysed using the McNemar test. For analysis of the impact of aspiration on outcome, data from both catheter systems were combined.

Results

No significant differences in age, number of collected oocytes, number of embryos or median embryo score were observed between the study group and control group (Table 1).

Biochemical and ongoing pregnancy rates in the study group patients were not significantly different to those observed in the control group (Table 1).

No discomfort or side effects of the aspiration were reported by any of the patients.

Discussion

The findings of this study indicate that the removal of endometrial secretions immediately prior to embryo transfer provides sufficient material for analysis of markers of receptivity without disrupting embryo implantation. This approach may overcome one of the barriers to the in-vivo investigation of endometrial receptivity in conception cycles: disruption of the process of implantation (Kolibianakis and Devroey, 2002; van der Gaast et al., 2002). While endometrial biopsy performed in IVF patients shortly after oocyte retrieval (Abate et al., 1987; Ubaldi et al., 1997) does not seem to have any adverse effects on implantation, a negative impact on
implantation was observed when the biopsy was taken immediately prior to embryo transfer.

Uterine flushing and uterine dialysis offer alternative techniques for obtaining endometrial material for analysis. Endometrial secretions can be analysed for functional markers of endometrial receptivity (Li et al., 1993a,b,c; 1998; Laird et al., 1997; Ledee-Bataille et al., 2002). However, animal studies suggest that uterine flushing may disrupt the endometrial epithelium (Milligan et al., 1984). To date, no case control studies have been carried out showing the impact of these techniques on pregnancy rates.

Beier and coworkers have demonstrated quantitative and qualitative changes of the protein patterns of endometrial secretion during the menstrual cycle (Beier and Beier-Hellwig, 1998; Beier-Hellwig et al., 1989, 1994). Analysis of the protein pattern is capable of indicating an adequate microenvironment in the uterine cavity, which in turn could facilitate the embryos attachment and implantation (Figure 1). This technique may therefore offer a useful alternative to histological evaluation of endometrial biopsies (Beier et al., 1994).

This study shows that aspiration of endometrial secretion, a simple and non-invasive technique, may be safely carried out immediately prior to embryo transfer without disrupting implantation. This study was powered to exclude a reduction of pregnancy rates from 30 to 10% per embryo transfer. However, in order to exclude a more subtle impact, a far larger study would have been required. Since a trend towards increased pregnancy rates after endometrial secretion removal was observed, it is unlikely that a larger sample size would have led to different conclusions. No adverse effects or side effects were reported. Indeed, this positive effect may be due to the removal of excessive cervical mucus prior to embryo transfer. However, the technique employed, whereby the catheter is clamped prior to removal from the cavity to prevent unintended aspiration of cervical mucus, renders this explanation unlikely. Recently, a pilot study showed that removal of ultrasonic visible fluid accumulation in the uterine cavity before the embryo transfer may have a beneficial effect on the implantation process (Griffiths et al., 2002). By reducing the volume of fluid in the uterine cavity aspiration of secretions prior to ET may facilitate the process of implantation, namely adhesion of the embryo to the endometrial surface.

In conclusion, endometrial secretion aspiration may provide a tool to assess endometrial receptivity in treatment and conception cycles (Beier-Hellwig et al., 1994), without compromising embryo implantation and establishment of clinical pregnancy. Prospective studies designed to further validate this approach to determining endometrial receptivity are now ongoing.

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