Smoking Decreases Endometrial Thickness in IVF/ICSI Patients

Rauchen verringert die Endometriumdicke bei IVF/ICSI-Patientinnen

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Key words
assisted reproduction (ART), infertility, menstrual cycle disorder, pregnancy, reproductive endocrinology

Schlüsselwörter
assistierte Reproduktionstherapie (ART), Infertilität, Zyklusstörung, Schwangerschaft, reproductive Endokrinologie

Results
Endometrial thickness was significantly lower in smoking patients than in non-smoking patients (10.4 ± 1.5 mm vs. 11.6 ± 1.8 mm). Age was significantly higher in women who failed to conceive. The total dose of gonadotropins administered was significantly lower in pregnant patients and the highest pregnancy rate was achieved with an rFSH protocol. BMI and number of cigarettes smoked did not influence treatment outcomes in this study.

Conclusion
We showed that smoking has a negative effect on endometrial thickness on the day of embryo transfer. This may help to further explain the detrimental influence of tobacco smoke on implantation and pregnancy rates during assisted reproduction therapy.

ABSTRACT

Introduction Smoking is a serious problem for the health care system. Many of the compounds identified in cigarette smoke have toxic effects on the fertility of both females and males. The purpose of this study was to determine whether smoking affects clinical factors during IVF/ICSI therapy in a single-center reproductive unit.

Material and Methods In a retrospective study of 200 IVF/ICSI cycles, endometrial thickness and the outcome of IVF/ICSI therapy were analyzed.
Introduction

The endometrium plays a crucial role for establishing and nourishing a healthy pregnancy. An undisturbed embryo-maternal dialogue is vital for successful implantation. One possible endocrine disruptor of a healthy uterine environment is maternal smoking during the time of assisted reproduction treatment (ART). Previously it has been shown that smoking can affect the outcome of IVF/ICSI treatments negatively. It can lead to reduced fertilization rates, pregnancy rates (PR) and live birth rates as well as to significantly higher chances of miscarriages or ectopic pregnancies [1].

So far, approximately 4000 compounds have been found in chemical analyses of tobacco smoke, including polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (BaP), nitrosamines, heavy metals (e.g. cadmium, lead, cobalt), alkaloids (nicotine), aromatic amines and carbonyl compounds [2]. The various compounds have different points of action in the female reproductive system. Nicotine seems to inhibit uterine decidualization as well as motility and migration of uterine endothelial cells in vitro [3]. Khorram et al. (2010) showed that nicotine and BaP in cigarette smoke inhibit endometrial epithelial cell proliferation through a nitric oxide-mediated pathway in a dose- and time-dependent manner [4]. BaP and other PAH derivatives alter cytochromes involved in estrogen metabolism, which could lead to a smoke-associated anti-estrogenic effect. Furthermore, smoking may also impair endometrial angiogenesis as both anti- and pro-angiogenetic factors have been found in cigarette smoke [1, 5]. Cadmium (Cd) can lead to reduced size or complete loss of follicles [6] as well as impaired cumulus expansion and progesterone synthesis in animal models [7] and human trophoblast cells in culture. A possible reason for decreased progesterone synthesis after cadmium exposure may be the dose-dependent decrease of LDL-receptor (LDL-R) expression, thereby impairing the first step of steroid synthesis, the internalization of cholesterol [8]. Highly dosed Cd also negatively affects the expression of the p450 side-chain cleavage (p450sccc) enzyme, which leads to reduced levels of estradiol [1]. Tsutsumi et al. (2009) found elevated prolactin (PRL) levels in human endometrial stromal cells treated with Cd, suggesting earlier decidualization [9]. The aim of this study was to determine the effect of maternal smoking on measurable clinical factors such as endometrial thickness, number of oocytes retrieved, number of mature oocytes, number of embryos transferred and dosage and length of hormonal stimulation during IVF/ICSI cycles.

Material and Methods

Patients

We retrospectively examined the data of 200 women undergoing assisted reproduction treatment between 2010 and 2011 in our clinic. Data were collected from lab records, ultrasound reports and patient intake questionnaires and included information about BMI, alcohol and cigarette consumption during time of treatment and infertility status. Institutional review board approval was obtained for the study from the Medical University Vienna ethics committee.
A significantly thinner endometrial stripe was measured in smokers than in non-smokers (10.4 ± 1.5 vs. 11.6 ± 1.8; p < 0.001). The maximum endometrial thickness measured in smoking patients was 14 mm, whereas a maximum of 16 mm was found in non-smoking patients.

In this setting smoking had no significant impact on the total number of oocytes retrieved, the number of mature oocytes, the length of hormonal stimulation and the total dose of gonadotropins administered (► Table 2). Interestingly, biochemical and clinical pregnancy rates were higher in smoking women. However, these results were not statistically significant (► Table 3). Smoking and non-smoking patients were similar in age and BMI, and the distribution of hormonal stimulation protocols did not differ significantly between the two groups.

### Table 1
Distribution of anamnestic parameters and cycle characteristics in biochemically pregnant and non-pregnant women.

<table>
<thead>
<tr>
<th>Variables/measurements</th>
<th>Pregnant (n = 69)</th>
<th>Non-pregnant (n = 131)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)(^a)</td>
<td>33.0 ± 4.8</td>
<td>36.0 ± 5.3</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>BMI (kg/m(^2))(^b)</td>
<td>23.3 ± 4.7</td>
<td>22.9 ± 4.4</td>
<td>0.663</td>
</tr>
<tr>
<td>No. of cigarettes (per day)(^b)</td>
<td>3.0 ± 6.0</td>
<td>1.8 ± 5.0</td>
<td>0.06</td>
</tr>
<tr>
<td>No. of oocytes retrieved(^b)</td>
<td>12.8 ± 8.0</td>
<td>9.0 ± 6.8</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>No. of mature oocytes(^b)</td>
<td>10.0 ± 7.5</td>
<td>6.8 ± 5.4</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>No. of embryos transferred(^b)</td>
<td>2.0 ± 0.5</td>
<td>2.0 ± 0.6</td>
<td>0.664</td>
</tr>
<tr>
<td>Endometrial thickness (mm)(^b)</td>
<td>11.3 ± 1.7</td>
<td>11.5 ± 1.9</td>
<td>0.438</td>
</tr>
<tr>
<td>Length of stimulation (days)(^b)</td>
<td>10.1 ± 1.6</td>
<td>10.1 ± 2.0</td>
<td>0.685</td>
</tr>
<tr>
<td>Total gonadotropin dose (IU)(^b)</td>
<td>2235.5 ± 750.5</td>
<td>2862.9 ± 1281.7</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

BMI: body mass index
\(^a\) T-Test; \(^b\) Mann-Whitney U-Test
* Statistically significant; data expressed as mean ± SD

### Table 2
Distribution of anamnestic parameters and cycle characteristics in smoking and non-smoking patients.

<table>
<thead>
<tr>
<th>Variables/measurements</th>
<th>Smokers (n = 35)</th>
<th>Non-smokers (n = 165)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)(^a)</td>
<td>35.0 ± 6.0</td>
<td>35.0 ± 5.2</td>
<td>0.955</td>
</tr>
<tr>
<td>BMI (kg/m(^2))(^b)</td>
<td>22.9 ± 4.3</td>
<td>23.0 ± 4.5</td>
<td>0.901</td>
</tr>
<tr>
<td>No. of oocytes retrieved(^b)</td>
<td>11.3 ± 8.8</td>
<td>10.1 ± 7.1</td>
<td>0.595</td>
</tr>
<tr>
<td>No. of mature oocytes(^b)</td>
<td>9.1 ± 8.1</td>
<td>7.7 ± 6.0</td>
<td>0.465</td>
</tr>
<tr>
<td>No. of embryos transferred(^b)</td>
<td>1.9 ± 0.6</td>
<td>2.0 ± 0.6</td>
<td>0.385</td>
</tr>
<tr>
<td>Endometrial thickness (mm)(^b)</td>
<td>10.4 ± 1.5</td>
<td>11.6 ± 1.8</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Length of stimulation (days)(^b)</td>
<td>10.2 ± 1.7</td>
<td>10.1 ± 1.9</td>
<td>0.878</td>
</tr>
<tr>
<td>Total gonadotropin dose (IU)(^b)</td>
<td>2536.6 ± 826.8</td>
<td>2669.7 ± 1224.5</td>
<td>0.958</td>
</tr>
</tbody>
</table>

BMI: body mass index
\(^a\) T-Test; \(^b\) Mann-Whitney U-Test
* Statistical significance; Data expressed as mean ± SD

### Table 3
Pregnancy and abortion rates in smoking and non-smoking patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Smokers* (n = 35)</th>
<th>Non-smokers (n = 165)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical pregnancy rate(^a)</td>
<td>48.6% (n = 17)</td>
<td>31.5% (n = 52)</td>
<td>0.054</td>
</tr>
<tr>
<td>Clinical pregnancy rate(^a)</td>
<td>37.1% (n = 13)</td>
<td>28.5% (n = 47)</td>
<td>0.31</td>
</tr>
<tr>
<td>Abortion rate(^a)</td>
<td>2.9% (n = 1)</td>
<td>4.2% (n = 7)</td>
<td>0.704</td>
</tr>
</tbody>
</table>

\(^a\) Chi-square test
* ≥ 1 cigarette smoked per day

**Influence of smoking**

A significantly thinner endometrial stripe was measured in smokers than in non-smokers (10.4 ± 1.5 vs. 11.6 ± 1.8; p < 0.001). The maximum endometrial thickness measured in smoking patients was 14 mm, whereas a maximum of 16 mm was found in non-smoking patients.

In this setting smoking had no significant impact on the total number of oocytes retrieved, the number of mature oocytes, the number of embryos transferred, the length of hormonal stimulation and the total dose of gonadotropins administered (► Table 2). Interestingly, biochemical and clinical pregnancy rates were higher in smoking women. However, these results were not statistically significant (► Table 3). Smoking and non-smoking patients were similar in age and BMI, and the distribution of hormonal stimulation protocols did not differ significantly between the two groups.
Discussion

We found a significantly thinner endometrium on the day of embryo transfer in women who actively smoked during IVF/ICSI treatment (p < 0.001). The possible molecular reasons for this phenomenon are diverse. The inhibition of uterine decidualization and of migration of uterine endothelial cells by nicotine and the impairment of endometrial epithelial cell proliferation by nicotine and BaP suggest a generally lower endometrial proliferation in smoking women. The anti-estrogenic effect of BaP and Cd [4, 5] possibly intensifies this tendency further. Moreover, Koukuras et al. demonstrated a close association between estrogen receptor polymorphism and the reduction of endometrial thickness after therapy with aromatase inhibitors [10]. Association studies for the estrogen receptor and the matrix metalloproteinase 9 gene have shown an association with smoking behavior [11]. Both genes are also involved in building endometrial layers [12].

To date, many authors have discussed the importance of measuring endometrial thickness as a predictor for successful implantation and pregnancy and subsequently came to contradictory conclusions [13]. While some were able to prove that a thicker endometrial stripe had a significant positive effect on pregnancy rates [14], others could not corroborate these results [15]. Moreover, some authors argued that a triple-lined endometrial pattern or endometrial volume [16] were better predictors than thickness. In a recent meta-analysis Weiss et al. did not find any evidence for an association between pregnancy rates and endometrial thickness during intrauterine insemination [17]. They concluded that cancelling IUI cycles due to thin endometrial thickness might negatively affect clinical care. However, there is a broad consensus that the probability of pregnancy is reduced when endometrial thickness is less than 6 mm [18].

Studies on the effect of cigarette smoke on IVF/ICSI treatment outcome have yet to reach conclusive results. Some authors have found significantly reduced implantation rates in active smokers [19, 20], while others failed to do so [21]. A study analyzing IVF outcome after oocyte donation from donors who smoked little or not at all found a significantly higher PR in non-heavy smokers (< 10 cigarettes/day) compared to heavy smokers (> 10 cigarettes/day), suggesting an impaired endometrial receptivity due to heavy smoking [22]. Interestingly, in our study, smoking women had a slightly higher biochemical and clinical pregnancy rate than non-smoking women. This result cannot be satisfactorily explained by the mean age or type of stimulation protocol used in smoking and non-smoking groups. In our opinion, it points to a more complex relationship between smoking, endometrial thickness and pregnancy rates. We found that the gonadotropins used for COH had a significant impact on the biochemical PR, as patients treated with rFSH alone had the highest PR (p < 0.001). A recently published randomized study by Miller et al. (2013) did not find a significant advantage for either rFSH or hMG [23], and Drakakis et al. (2002) reported a higher fertilization rate in patients treated with highly purified FSH (pFSH) but not a higher PR [24]. Further studies are necessary to better understand the difference in the performance of these hormones. In our study, the total dose of administered gonadotropins was significantly lower in patients who were able to conceive (p < 0.001). This may well be due to the fact that women with a higher age (over 35 years) who had a lower PR (p < 0.001) also needed higher doses of gonadotropins (p < 0.001). Conversely, this means that the younger the women, the higher the PR and the lower the total dose of hormones required. Sharif et al. (1998) reported similar results [25]. Length of stimulation, however, did not differ between pregnant and non-pregnant patients. Furthermore, our results confirm the detrimental effect of increased maternal age on ART outcome found in other studies [26]. Mean patient age was significantly lower in the pregnant group (p < 0.001), and patients under 35 years of age had a significantly higher biochemical PR than those aged 35 or higher (p < 0.001). We also found significantly higher numbers of oocytes in the pregnant group (p < 0.001), which confirms the findings of Dor et al. (1992) [27]. By contrast, other studies showed that while a low number of oocytes (< 5) significantly reduced PR, a high number (> 15) did not affect the success rate but rather increased the risk of ovarian hyperstimulation syndrome [28, 29]. The transfer of good quality embryos seems to be more important than the total number of oocytes retrieved [30].

This study is one of very few published studies investigating the effects of cigarette smoke on endometrial thickness on the day of ET during ART cycles with a rFSH, hMG or rFSH + hMG protocol. We were able to show that smoking decreases the endometrial thickness. However, this was not correlated with pregnancy rates in our study. We are aware of the fact that in this study having different ultrasonography examiners might be a source of bias. This can be overcome by using 3D-sonography, which offers a better possibility of standardization and reduces the influence of the physician. In addition, recall bias might also influence the study outcome. A limitation of this study is the fact that the number of women who smoked was low. A prospective study would be helpful to underline the validity of our study. Further examinations, preferably with a bigger population, are needed to better understand the clinical effects of smoking on the female reproductive system during IVF/ICSI therapy.

Conclusion for Clinical Practice

It is widely accepted that cigarette smoking negatively affects IVF and ICSI outcomes. More prospective studies with bigger study populations are necessary to understand this relationship better and to allow clinicians to educate their patients more about the risks of tobacco smoke and the impact on the success of treatment. Nevertheless, it is always recommended to advise patients planning to undergo ART to reduce or better yet, to cease cigarette smoking prior to ovarian hyperstimulation.

Conflict of Interest

The authors declare that there are no conflicts of interest in connection with this article.
References


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