

# 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, reproduktive Endokrinologie

received 4.9.2017

revised 15.11.2017

accepted 26.11.2017

### Bibliography

DOI <https://doi.org/10.1055/s-0043-123762>

Geburtsh Frauenheilk 2018; 78: 78–82 © Georg Thieme  
Verlag KG Stuttgart · New York | ISSN 0016-5751

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### 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.

**Results** Endometrial thickness was significantly lower in smoking patients than in non-smoking patients ( $10.4 \pm 1.5$  mm vs.  $11.6 \pm 1.8$  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.

### ZUSAMMENFASSUNG

**Einleitung** Rauchen ist ein ernsthaftes Problem für das Gesundheitssystem. Viele der im Zigarettenrauch nachgewiesenen Verbindungen haben toxische Wirkungen auf die Fruchtbarkeit von Frauen und Männern. Ziel dieser Studie war es zu untersuchen, ob Rauchen klinische Parameter während einer IVF/ICSI-Therapie beeinflusst.

**Material und Methoden** In einer retrospektiven Studie mit 200 IVF/ICSI-Zyklen wurden die Endometriumdicke und verschiedene Zielparameter der IVF/ICSI-Therapie analysiert.

**Ergebnisse** Die Dicke der Gebärmutter Schleimhaut war bei rauchenden Patienten signifikant geringer als bei Nichtrauchern ( $10,4 \pm 1,5$  mm vs.  $11,6 \pm 1,8$  mm). Das Alter war bei Frauen, die nicht schwanger waren, deutlich höher. Die Gesamtdosis der verabreichten Gonadotropine war bei schwangeren Patienten signifikant niedriger und die höchste Schwangerschaftsrate wurde mit einem rFSH-Protokoll festgestellt. BMI und die Anzahl der gerauchten Zigaretten hatten keinen Einfluss auf das Behandlungsergebnis in dieser Studie.

**Fazit** Wir konnten zeigen, dass Rauchen die Dicke der Gebärmutter Schleimhaut am Tag des Embryotransfers negativ beeinflusst. Diese Studie kann daher beitragen, den schädlichen Einfluss des Tabakrauchs auf die Implantations- und Schwangerschaftsrate während der assistierten Reproduktionstherapie zu erklären.

## 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-angiogenic 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 (p450scc) 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.

### Methods

Controlled ovarian hyperstimulation (COH) was achieved by the daily administration of recombinant FSH (rFSH) and/or human menopausal gonadotropin (hMG) in different dosages. The exact dose and length of stimulation was adjusted according to hormone levels and the number of follicles measured by various ultrasound examinations during the time of COH. Endometrial thickness was measured by different examiners using two-dimensional transvaginal ultrasound in the midsagittal plane of the uterus, from the myometrial endometrial junction through the thickest part to the outer edge of the endometrium on the day of embryo transfer (ET). Biochemical pregnancy was determined by  $\beta$ -hCG levels while clinical pregnancy was determined by fetal heart activity six weeks after ET.

### Statistics

Statistical analysis was performed using SPSS version 18 software (SPSS Inc., Chicago, IL, USA). Student's t-test was used for normally distributed continuous variables and Mann-Whitney test was used for skewed data. The chi-square test was used for categorical variables. A Bonferroni correction was performed, and a two tailed p-value of  $<0.001$  was considered statistically significant. Results are expressed as mean  $\pm$  SD.

## Results

### Descriptive parameters

Patients' age ranged from 22 to 47 years ( $35.0 \pm 5.3$ ); the women's mean BMI was  $23.0 \pm 4.5$ . An ICSI was carried out in 80% of cases ( $n = 160$ ), while for 20% the method of choice was IVF ( $n = 40$ ). The biochemical pregnancy rate was 34.5% ( $n = 69$ ), the clinical pregnancy rate was 30.0% ( $n = 60$ ), and eight pregnancies ended in miscarriage (4.0%). Endometrial thickness on the day of embryo transfer (ET) ranged from 5 mm to 16 mm ( $11.4 \pm 1.8$  mm). 17.5% of women were smokers at the time of treatment ( $n = 35$ ).

### Characteristics of pregnant and non-pregnant patients

All cycle characteristics of pregnant and non-pregnant cases are shown in ► **Table 1**. Maternal age was significantly lower in patients who conceived than in those who failed to do so ( $33.0 \pm 4.8$  vs.  $36.0 \pm 5.3$ ;  $p < 0.001$ ). The number of oocytes retrieved was significantly higher in the biochemically pregnant group than in the non-pregnant group ( $12.8 \pm 8.0$  vs.  $9.0 \pm 6.8$ ;  $p < 0.001$ ) as was the number of mature oocytes ( $10.0 \pm 7.5$  vs.  $6.8 \pm 5.4$ ;  $p < 0.001$ ). Women receiving only rFSH for stimulation had the highest PR (44.4%;  $p < 0.001$ ) compared to those receiving either only hMG or a combination of both. The total dose of gonadotropins administered for COH was significantly lower in patients who succeeded in establishing a biochemical pregnancy ( $2235.5 \pm 750.5$  IU vs.  $2862.9 \pm 1281.7$  IU;  $p < 0.001$ ). BMI, number of cigarettes smoked per day, number of embryos transferred, endometrial thickness and length of stimulation did not significantly differ between the pregnant and non-pregnant groups.

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

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

BMI: body mass index

<sup>a</sup> T-Test; <sup>b</sup> 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.

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

BMI: body mass index

<sup>a</sup> T-Test; <sup>b</sup> Mann-Whitney U-Test

\* Statistical significance; Data expressed as mean ± SD

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

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

<sup>a</sup> 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.

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