

Cigarette smoking affects uterine receptiveness

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BACKGROUND: Cigarette smoking has long been known to have an effect on female fertility. The existence of an ovarian factor is clear when one considers that the mean age of the menopause is lower and IVF cycle outcome is worse in heavy smokers. The hypothesis of a concomitant uterine effect is raised by indirect evidence from *in vitro* and *in vivo* studies, but as yet, no direct evidence has been gained to confirm its existence. In this work, we analyse the association between smoking habit in oocyte recipients and cycle outcome. **METHODS:** We have retrospectively analysed the outcome of all oocyte donation cycles performed in our clinic from January 2002 to June 2005 from which there was available information regarding patient current smoking status. Husband and donor smoking status were controlled variables, as well as donor and recipient age, patient body mass index, embryo number and quality and duration of endometrial priming. **RESULTS:** Pregnancy rate (PR) in non-heavy smokers (0–10 cigarettes/day) was significantly higher than in heavy smokers (>10 cigarettes/day) (52.2 versus 34.1%, respectively). Interestingly, multiple PR was significantly higher in heavy smokers (60 versus 31%). **CONCLUSION:** Tobacco consumption determines reduced uterine receptiveness and an increased risk of multiple pregnancies. This last issue remains to be clarified.

Key words: smoking/oocyte donation/uterine effect/pregnancy/multiple pregnancy

Introduction

Cigarette smoking has long been known to have an effect on female fertility. The association between tobacco consumption and subfecundity in natural cycles is consistently reported in epidemiological studies (Augood *et al.*, 1998). The effect of smoking on the ovaries is clear when one considers that the mean age of the menopause in smokers is lower than in non-smokers (Jick *et al.*, 1977). Comparisons of IVF cycles in heavy smokers (most frequently defined as consumers of >10 cigarettes per day) and non-heavy smokers (0–10 cigarettes a day) confirm tobacco consumption's effects on ovarian function: higher basal FSH levels and cancellation rates, lower mean number of retrieved oocytes and higher miscarriage rate (MR) and poorer implantation, ongoing pregnancy and live birth rates have all been reported (Feichtinger *et al.*, 1997; Augood *et al.*, 1998; El-Nemr *et al.*, 1998; Zenzes, 2000; Crha *et al.*, 2001; Klonoff-Cohen *et al.*, 2001; Lintsen *et al.*, 2005).

The hypothesis of a concomitant uterine effect has emerged from *in vitro* studies in which loss of cell adhesion and reduced basement membrane invasion have been observed in human RL95-2 endometrial adenocarcinoma cells exposed to tobacco constituents [namely benzo(a)pyrene] (Shiverick and Salafia, 1999). This cell line serves as an *in vitro* model for the receptive endometrium because of its adhesiveness for trophoblast cells. In addition, some authors have reported lower pregnancy rate (PR) and implantation rate (IR) in smokers undergoing

IVF cycles in which the number of morphologically good embryos replaced has been similar to that in non-smokers (Neal *et al.*, 2005). This suggests that altered uterine receptiveness may be a factor in the former group of women, although reduced implantation could also be because of embryonic factors that belie a good morphology on day 3 of development (Zenzes, 2000). Finally, literature on the effect of cigarette smoking on endometrial blood flow reports a significantly lower endometrial and subendometrial vascularity and flow intensity throughout the normal menstrual cycle in smokers (Raine-Fenning *et al.*, 2004), although no such effect has been detected during IVF treatments (Ng *et al.*, 2006). As in many other issues, oocyte donation (OD) is the model that most objectively ascertains the presence of a uterine factor in the outcome of assisted reproduction cycles in smokers. To the best of our knowledge, no studies exploring this model have yet been published. In this report, we analyse the association between a smoking habit in oocyte recipients and cycle outcome in 785 embryo transfer cycles.

Materials and methods

Our study group comprises all the OD cycles performed at the IVI-Valencia from January 2002 to June 2005, which fulfilled the following inclusion criteria: availability of information regarding patient current smoking status, non-heavy-smoking oocyte donor (0–10 cigarettes

a day), non-smoking husband and embryo transfer performed on day 3 of development. None of the donors were heavy smokers (>10 cigarettes per day) because of the aforementioned association between heavy smoking and poorer rates of ovarian response and pregnancy (El-Nemr *et al.*, 1998; Crha *et al.*, 2001; Klonoff-Cohen *et al.*, 2001). Because smoking ≤ 10 cigarettes per day has not been associated with IVF results inferior to those achieved with non-smokers (El-Nemr *et al.*, 1998; Crha *et al.*, 2001; Klonoff-Cohen *et al.*, 2001), cycles with donors who smoked ≤ 10 cigarettes a day were maintained in this study. Husband smoking status was also controlled, as subfecundity in natural cycles and poorer IVF outcome have been reported in non-smoking women whose husbands are smokers (Hassan and Killick, 2004; Neal *et al.*, 2005). Because one of these studies has questioned the minimal number of cigarettes/day associated with reduced PR in passive smokers (Neal *et al.*, 2005), we preferred to include only those cycles performed in couples in which the husband was a non-smoker. Only first-cycle recipients were included, as the results of consecutive cycles in the same patient may lead to an overestimation of outcomes. Owing to the retrospective nature of this study and the complete anonymity of the participants, the approval of the Institutional Review Committee was not necessary.

Oocyte donors

All donors were included in our OD programme after being thoroughly informed and having fulfilled our inclusion criteria. Subjects were aged between 18 and 35 years. We had access to their complete medical history, which included current or past exposure to radiation or hazardous chemical substances, IV drug use and reproductive history. All subjects were shown to be normal after a physical and gynaecological examination and were confirmed as having no family background of hereditary or chromosomal diseases. In addition, they all had a normal karyotype and tested negative in a screening for sexually transmitted diseases (STDs).

The patients underwent a prolonged protocol of down-regulation with daily doses of a GnRH agonist (GnRH-ag). Transvaginal ultrasound was performed to ascertain ovarian quiescence on the first 3 days of menses, after which controlled ovarian stimulation was initiated. In short, the starting dose varied from 150 to 300 U/day of FSH and/or human menopausal gonadotrophin for the first 2–5 days, according to age, body mass index (BMI) and response to previous ovarian stimulations. The dose was then adjusted according to ovarian response, which was monitored every 2–3 days through serum E2 levels and ultrasound. Stimulation was carried out until leading follicles reached a mean diameter of >18 mm. Human chorionic gonadotrophin was then administered, and ovarian puncture was performed 36 h later.

Anonymous donors were matched with their recipient(s) according to phenotype and blood group.

Oocyte recipients

Oocyte recipients were considered for inclusion in our OD programme because of one of the following diagnoses: premature ovarian failure/menopause, failure to achieve pregnancy after at least three cycles of assisted reproduction techniques, genetic or chromosomal disorders, low response to controlled ovarian hyperstimulation or recurrent miscarriage.

All oocyte recipients were undergoing hormone replacement therapy (HRT), as previously described (Remohí *et al.*, 1995). In patients with ovarian function, a depot GnRH-ag was administered in the mid-luteal phase of the cycle. HRT was initiated on days 1–3 of the following cycle, doses of estradiol valerate (Progynova, Schering Spain, Madrid, Spain) in doses that increased as follows: 2 mg/day for the first 8 days of treatment, 4 mg/day for the following 3 days and

6 mg/day until a pregnancy test was performed after embryo transfer or until vaginal spotting was identified before transfer, in which case the cycle was cancelled and excluded from the study. On days 15–16 of HRT, a transvaginal ultrasound was performed to measure endometrial thickness and serum E2 levels were tested. Recipients without ovarian function underwent the same endometrial preparation protocol, except for the administration of the depot GnRH-ag.

Micronized progesterone (800 mg/day, vaginally) (Progeffik, Effik Laboratories, Madrid, Spain) administration was initiated the day after OD, and embryos were transferred on day 3 of embryo cleavage.

Embryos were classified according to cell number, symmetry and degree of fragmentation (Alikani *et al.*, 2000). Serum α -hCG was measured 14 days after oocyte retrieval. Clinical pregnancy was diagnosed 1–2 weeks later if the existence of an intrauterine gestational sac was confirmed by transvaginal ultrasound. PR was defined as the percentage of patients undergoing embryo transfer who were shown to have one or more gestational sacs on ultrasound evaluation. IR was obtained by dividing the number of gestational sacs seen during ultrasound by the number of replaced embryos. The number of embryos to be replaced was decided based on the following criteria: embryo quality, patient age, previous assisted reproduction treatment outcomes, reproductive history and the presence of uterine malformations. MR was defined as the percentage of pregnancies that terminated before the completion of the 20th week of gestation following prior ultrasound detection of the embryo's heartbeat.

Statistical analysis

With the goal of confirming that light smoking does not affect the quality of ART cycles either through an oocyte or through a uterine factor, we have compared the outcome of cycles with non-smoking and light-smoking (1–10 cigarettes a day) oocyte donors, as well as cycles with non-smoking and light-smoking oocyte recipients.

Subsequently, cycles were divided into two other groups according to oocyte recipients' smoking status only: group 1 was composed of cycles performed to non-heavy smokers (between 0 and 10 cigarettes a day) and group 2 was composed of cycles performed to heavy smokers (>10 cigarettes a day). These cycles were so compiled to answer the main question of this study: Does heavy smoking affect uterine receptiveness?

Student's *t*-test was used to compare groups with respect to recipient and donor age, recipient BMI, duration of endometrial priming, mean number of embryos replaced and mean number of good-quality embryos replaced.

The effect of a smoking habit on PR, IR, MR and multiple pregnancy rate (MPR) was evaluated by the non-parametric chi-square test.

Results

The entire series studied comprised 785 OD cycles. Table I summarizes the characteristics and the outcome of cycles with non-smoking and light-smoking oocyte donors ($n = 785$). The only statistically significant difference observed between groups was that light smokers were younger than non-smokers (24.9 versus 26.0 years, respectively; $P < 0.0001$).

Table II summarizes the characteristics and the outcome of cycles with non-smoking and light-smoking oocyte recipients ($n = 741$). None of the parameters evaluated were significantly different between groups.

In Table III, we can see the characteristics of groups 1 and 2. Group 1 gathered the 741 cycles performed to non-smoking and light-smoking oocyte recipients, and group 2 included 44

Table I. Group characteristics and outcome of cycles with non-smoking and light-smoking oocyte donors*

	Non-smokers	Light smokers
Number of oocyte donation cycles	401	384
Recipient age (years) ^a	39.9 ± 5.1	39.3 ± 4.9
Recipient BMI (kg/m ²) ^a	21.9 ± 5.4	22.4 ± 5.4
Duration of endometrial priming (days) ^a	33.2 ± 11.3	33.7 ± 11.4
Number of embryos replaced ^a	2.1 ± 0.4	2.1 ± 0.5
Number of good-quality embryos replaced ^a	1.7 ± 0.6	1.7 ± 0.5
Donor age (years) ^b	26.0 ± 4.3	24.9 ± 4.3
Non-smoking recipient (%) ^a	86.5 (347/401)	86.7 (333/384)
Light-smoking recipient (%) ^a	7.7 (31/401)	7.8 (30/384)
Heavy-smoking recipient (%) ^a	5.7 (23/401)	5.5 (21/384)
Pregnancy rate (%) ^a	49.9 (200/401)	52.6 (202/384)
Implantation rate (%) ^a	32.0 (263/822)	33.7 (271/805)
Miscarriage rate (%) ^a	17.0 (34/200)	13.4 (27/202)
Multiple pregnancy rate (%) ^a	31.0 (62/200)	33.2 (67/202)

*Where applicable, values are expressed as mean ± SD.

^aP = non-significant.

^bP < 0.0001.

Table II. Group characteristics and outcome of cycles with non-smoking and light-smoking oocyte recipients*

	Non-smokers	Light smokers
Number of oocyte donation cycles	680	61
Recipient age (years) ^a	39.7 ± 5.1	38.8 ± 3.8
Recipient BMI (kg/m ²) ^a	22.2 ± 5.4	21.6 ± 5.9
Duration of endometrial priming (days) ^a	33.5 ± 11.4	31.2 ± 10.1
Number of embryos replaced ^a	2.1 ± 0.4	2.1 ± 0.4
Number of good-quality embryos replaced ^a	1.7 ± 0.6	1.7 ± 0.7
Non-smoking donor (%) ^a	51.0 (347/680)	50.8 (31/61)
Light-smoking donor (%) ^a	49.0 (333/680)	49.2 (30/61)
Donor age (years) ^a	25.5 ± 4.3	25.0 ± 4.1
Pregnancy rate (%) ^a	51.6 (351/680)	59.0 (36/61)
Implantation rate (%) ^a	32.3 (461/1428)	38.3 (49/128)
Miscarriage rate (%) ^a	16.5 (58/351)	5.5 (2/36)
Multiple pregnancy rate (%) ^a	30.5 (107/351)	36.1 (13/36)

*Where applicable, values are expressed as mean ± SD.

^aP = non-significant.

Table III. Group characteristics and outcome of cycles with non-heavy-smoking (group 1) and heavy-smoking (group 2) oocyte recipients*

	Group 1	Group 2
Number of oocyte donation cycles	741	44
Recipient age (years) ^a	39.6 ± 5.0	38.9 ± 4.9
Recipient BMI (kg/m ²) ^a	22.1 ± 5.4	22.2 ± 4.9
Duration of endometrial priming (days) ^a	33.3 ± 11.3	35.2 ± 11.3
Number of embryos replaced ^a	2.1 ± 0.4	2.1 ± 0.4
Number of good-quality embryos replaced ^a	1.7 ± 0.6	1.8 ± 0.5
Donor age (years) ^a	25.5 ± 4.3	25.8 ± 4.5
Pregnancy rate (%) ^b	52.2 (387/741)	34.1 (15/44)
Implantation rate (%) ^a	33.2 (510/1534)	25.8 (24/93)
Miscarriage rate (%) ^a	15.5 (60/387)	6.7 (1/15)
Multiple pregnancy rate (%) ^b	31 (120/387)	60 (9/15)
Twins (%) ^a	97.5 (117/120)	100 (9/9)
Triplets (%) ^a	2.5 (3/120)	0 (0/9)

*Where applicable, values are expressed as mean ± SD.

^aP = non-significant.

^bP = 0.02.

cycles to heavy-smoking oocyte recipients. No differentiation was made between non-smoking or light-smoking donor or recipient, on the basis of the results seen on Tables I and II.

Recipient and donor age, recipient BMI, duration of endometrial priming, mean number of embryos replaced and mean number of good-quality embryos replaced were almost identical in both groups.

With respect to OD cycle outcome, PR was significantly higher in group 1 (non-heavy smokers) than in group 2: 52.2 versus 34.1%, respectively ($P = 0.02$) (Table III). The IR also appeared higher in group 1 (33.2 versus 25.8%), but this difference was not statistically significant. The same was true when MR was compared in the two groups. Interestingly, the MPR was significantly higher in group 2: 60% of gestations observed in this group were twins, compared with a MPR of 31% ($P = 0.02$) seen in group 1 (Table III).

Discussion

The negative impact of cigarette smoking on reproductive function is considerable. Acceleration of ovarian reserve depletion in smokers was confirmed when the first successful IVF cycle was reported (Jick *et al.*, 1977; Steptoe and Edwards, 1978). Since then, there have been reports of alterations in parameters of ovarian response in IVF cycles performed in heavy smokers (El-Nemr *et al.*, 1998; Crha *et al.*, 2001; Klonoff-Cohen *et al.*, 2001). A higher ectopic PR has also been reported in such patients, and studies in animal models have confirmed that tobacco alters tubal function (Knoll and Talbot, 1998; Saraiya *et al.*, 1998). Even cervical factor infertility has been associated with smoking (Phipps *et al.*, 1987). Furthermore, male smokers have an increased percentage of chromosomally abnormal spermatozoa (Rubes *et al.*, 1998) and poorer classical sperm parameters (count and morphology) (Sharpe and Franks, 2002), although the latter findings are somewhat controversial. Finally, deleterious changes in the placenta and fetus are more frequent in pregnant smokers who present higher rates of low birthweight and perinatal and neonatal mortality (Walsh, 1994). Despite these data, and to the best of our knowledge, no studies of the impact of smoking on OD outcome have been published until now. This is highly relevant, as OD is the only model that allows an appropriate evaluation of uterine receptiveness.

In this retrospective study, we have confirmed that light smoking has no significant impact on ART cycles either through an oocyte or through a uterine factor (Tables I and II). Most relevantly, heavy smokers were shown to have a significantly lower probability of becoming pregnant through OD (Table III). These patients also displayed a significantly higher twin gestation rate.

Whenever possible, biochemical assessment of smoking by-products in blood is advisable to validate the information provided by a patient with respect to her tobacco consumption. In practice, many of the studies related to this matter are retrospective, which means that self-reporting is relied on. Self-reporting of smoking habits has been validated by a meta-analysis of published reports in which its sensitivity and specificity has been shown to be high (Patrick *et al.*, 1994).

Studies of a harmful exposure in humans must nearly inevitably be observational. In such studies, potentially confounding interactions are controlled through data depuration. The high number of OD cycles performed in our institution provides us

with an adequate sample size even after data depuration has been carried out. Thus, we could limit our analysis to cycles in which heavy-smoking oocyte donors were excluded and husbands were all non-smokers. Other variables that may interfere with OD outcome have been controlled (Table III). The effect of caffeine and alcoholic consumption on fertility is controversial, with many studies failing to isolate these factors and/or demonstrate their relevance (Klonoff-Cohen, 2005). Nevertheless, in our study, none of the heavy-smoking patients (group 2) who failed to get pregnant consumed more than four drinks per week, and only one consumed more than 250 mg/day of caffeine, both of which are limits recommended for patients concerned about their fertility potential (Barbieri, 2001). With respect to our findings, the significantly lower PR seen in heavy smokers (34.1 versus 52.2%) demonstrates that cigarette smoking negatively affects uterine receptiveness independently from its effect on ovarian function. Comparison between our data and previous literature has not been possible because of the lack of similar studies. That said, information available before the performance of our study highlights a possible association between cigarette smoking and reduced uterine receptiveness. Increased resting uterine tonus in female smokers was described long ago (Neri and Eckerling, 1969). Also, human endometrial carcinoma cells that usually adhere to trophoblast cells have been demonstrated to lose cell adhesion and display reduced basement membrane invasion when exposed *in vitro* to tobacco constituents (Shiverick and Salafia, 1999). These reports suggest that uterine function is altered in several ways, either mechanically or biochemically. When considering the mechanisms through which tobacco constituents may alter uterine function, an insight into its effect on ovarian tissue is useful.

Among all reproductive system targets, ovarian tissue is by far the most widely studied in terms of the consequences of exposition to tobacco compounds. In human and animal models, *in vivo* and *in vitro* studies on ovarian cell types (the oocyte, granulosa, luteal and endothelial cells) have demonstrated that smoke compounds affect physiological processes by altering the activation of the apoptotic pathway and the production, release and action of enzymes, hormones, growth factors and cytokines, by impairing cell division and through direct cell toxicity (Barbieri *et al.*, 1986; Bernstein *et al.*, 1989; Blackburn *et al.*, 1994; Magers *et al.*, 1995; Zenzes *et al.*, 1995; Jordan *et al.*, 1998; Villablanca, 1998; Shiverick and Salafia, 1999; Vrsanska *et al.*, 2003; Mlynarcikova *et al.*, 2005; Bordel *et al.*, 2006).

When we extrapolate all these mechanisms to the uterine environment, the possible consequences in both the endometrium and the embryo are easy to imagine. Their own homeostasis may be disrupted, as well as their complex molecular interactions.

The other significant finding of our study was the increased incidence of MPR (twin gestations) among the heavy smokers who became pregnant. Indeed, the reduction in IR seen in heavy-smoking oocyte recipients did not reach statistical significance because of the increased rate of twin gestations. In other words, if MPR in heavy smokers had been the same as that observed in non-heavy smokers, IR reduction would have been statistically significant. The increased MPR observed in

our study in heavy smokers is of great interest. The association of reduced PR and increased MPR suggests a paradoxical effect of tobacco constituents, with some women displaying impaired implantation and others presenting the opposite phenomenon. A high MPR in heavy smokers has previously been described in spontaneous pregnancies (Parazzini *et al.*, 1996; Olsen *et al.*, 1998). Mechanisms that could explain this association (alterations in estrogens and gonadotrophin concentrations leading to polyovulation and dizygotic twins, or zona pellucida hardening leading to embryo splitting during hatching and monozygotic twins) do not explain the same finding in OD cycles. All our group 2 twin gestations were dizygotic, as expected. These results need to be confirmed by an analysis of a higher number of pregnancies in heavy smokers undergoing OD. In any case, it is interesting that paradoxical dose-dependent effects of tobacco constituents have also been described in ovarian tissue. In the ovary, high doses of nicotine can inhibit endothelial cell division and promote cell death, whereas at lower concentrations this alkaloid stimulates endothelial cell DNA synthesis and cell proliferation (Villablanca, 1998). Also, high cadmium concentrations inhibit p450_{sc} transcriptional activity in the ovary, whereas low concentrations stimulate p450_{sc} transcriptional activity (Mlynarcikova *et al.*, 2005). In the case of molecules that act by binding to receptors, their effect depends not only on their concentration but also on the concentration of their receptors in a certain tissue and the cellular environment as a whole (Bordel *et al.*, 2006). Therefore, the same molecular concentration may result in opposite effects in different individuals. If tobacco compounds trigger this kind of paradoxical response in the endometrial tissue and/or the embryo itself, resulting in the prevalence at times of pro-implantation cytokines and growth factors and at other times of anti-implantation molecules, a reduced general PR and an increased MPR may be jointly observed. In addition to the confirmation of our findings in a higher number of OD cycles in heavy smokers, it would be enlightening to study differences in gene expression in the endometrium of heavy smokers who fail to get pregnant after OD and in those whose cycles culminate in a twin gestation.

Our study found no significant difference between the MR of heavy-smoking and non-heavy-smoking oocyte recipients. There is substantial evidence to indicate that smoking increases the risk of miscarriage by over one-third in spontaneous pregnancies (Walsh, 1994). The same is true for MR in IVF cycles (Klonoff-Cohen, 2005). OD differs from these situations in that the ovarian factor (oocyte quality and granulosa/luteal cells function) is absent, and adequate sex steroid support is guaranteed by the exogenous administration of estrogen and progesterone. Nevertheless, the number of pregnancies achieved in heavy-smoking oocyte recipients in our study is clearly insufficient for measuring the effect of this habit on miscarriage risk because of uterine factor.

In conclusion, tobacco consumption determines a reduced uterine receptiveness. Patients should be duly informed about the implications of this finding for the outcome of natural and IVF cycles, and particularly OD cycles. The question of the increased incidence of MPR among smokers because of a uterine factor remains to be clarified.

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