The effect of female tobacco smoking on IVF outcomes

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BACKGROUND: Cigarette smoking is widely believed to be associated with decreased fecundity in naturally conceiving populations; however, the effect of female smoking on pregnancy outcomes in patients undergoing IVF is unclear. METHODS: A retrospective analysis of 389 consecutive patients undergoing first cycle IVF was performed. Outcomes of peak estradiol (E_2) levels, log mean ovarian volume, number of oocytes retrieved, oocyte maturity in ICSI, fertilization rate, cleavage rate, embryo quality, percentage of high-quality embryos, pregnancy and live birth were assessed in patients reported as never smokers, past smokers and current smokers. Potential confounding variables evaluated included day 3 FSH, number of oocytes retrieved, embryo quality, caffeine and alcohol consumption. The population was also stratified by female age (<35 and ≥35 years). RESULTS: A total of 9.3% of our patients reported current smoking and 12.1% reported a history of smoking. Smoking status did not significantly affect pregnancy outcome, live birth rate or any other indicated outcome. CONCLUSIONS: A total of 21.4% of IVF patients in this study had past or present exposure to cigarette smoking with no measurable effect on IVF outcome.

Key words: cigarette smoking/infertility/IVF

Introduction

Tobacco use is widely recognized as a significant health hazard, and smoking in reproductive aged women has been associated with decreased fecundity and increased time to conception in many epidemiologic studies (Baird and Wilcox, 1985; Hughes and Brennan, 1996; Hull *et al.*, 2000). Overall, the literature supports a small but clinically significant detrimental effect of female smoking on time to conception, although some studies have demonstrated no effect, or an effect only in heavy smokers (Harlap and Baras, 1984). Although the time to natural conception may be delayed in smokers, overall cumulative pregnancy rates have been reported to be remarkably similar (Laurent *et al.*, 1992).

The ability to accurately identify the precise effect of smoking on fertility is difficult because of multiple confounding factors, which may themselves contribute to diminished fertility, such as socioeconomic status, caffeine and alcohol consumption. The exact mechanism by which smoking might diminish fertility is unknown, although toxins in cigarette smoke have been implicated in diminishing ovarian reserve (Zenzes, 2000). One study which measured cotinine, the major metabolite of nicotine, in oocyte follicular fluid demonstrated lower fertilization rates in oocytes with cotinine concentration >20 ng/ml (Rosevear *et al.*, 1992). Some investigators report that smoking is associated with lower circulating estradiol (E_2) levels, suggesting that smoking negatively impacts ovarian function

(Van Voorhis *et al.*, 1992); however, others demonstrate no difference in E_2 levels (Hughes *et al.*, 1994; Sterzik *et al.*, 1996). Smoking is associated with an earlier age of menopause onset, suggesting that it may exacerbate the steady decrease in ovarian reserve that occurs with advancing female age (Jick and Porter, 1977).

The possible effect of smoking on ovarian reserve is of particular concern to women undergoing treatment for infertility. Although it is tempting to extrapolate the apparent effect of smoking on naturally conceiving women to a similar effect on women undergoing assisted conception, in actuality these two groups are quite dissimilar. Artificial reproductive technologies may render the gametes less susceptible to toxins in cigarette smoke, and it is possible that other underlying infertility factors may overshadow any small deleterious effect of smoking.

The impact of paternal smoking on fertility is widely reported to be detrimental. Sofikitis *et al.* (1995) reported that morphologic sperm abnormalities may be the cause of impaired sperm fertilizing capacity in smokers. Animal models have supported an impaired epididymal sperm maturation process and impaired fertilization rate in current smokers undergoing IVF, but not ICSI (Kapawa *et al.*, 2004)

Studies evaluating the effect of female smoking on couples undergoing assisted conception have yielded conflicting results. Some studies have demonstrated similar clinical pregnancy rates among smokers and non-smokers (Trapp *et al.*,

1986; Hughes *et al.*, 1994). Other studies report similar fertilization rates, but a reduction in clinical pregnancy rate (Harrison *et al.*, 1990; Pattinson *et al.*, 1991). Still others report a decline in fertilization rates (Elenbogen *et al.*, 1991; Rosevear *et al.*, 1992; Rowlands *et al.*, 1992). Some studies also report that part of the decline in clinical pregnancy rate is attributable to an increased rate of spontaneous abortion in smokers (Pattinson *et al.*, 1991). Neal *et al.* (2005) reported a decline in implantation rates among smokers despite similar embryo quality between mainstream, sidestream and non-smokers. A meta-analysis of observational studies of subfertile women undergoing IVF yielded an odds radio of 0.66 (95% CI = 0.49–0.88) for pregnancies per number of IVF-treated cycles in smokers versus non-smokers; however, the authors acknowledge many sources of bias, including publication bias (Augood *et al.*, 1998).

Given the uncertain conclusions about the effect of smoking on women undergoing assisted conception, we designed a large retrospective cohort study to evaluate the effect of smoking on peak E_2 levels, log mean ovarian volume, number of oocytes retrieved, oocyte maturity in ICSI, fertilization rate, cleavage rate, embryo quality, percentage of high-quality embryos, pregnancy and live birth in an IVF population at an academic medical centre. We hypothesized that smoking would have a negative effect on these parameters and that this effect would be compounded by age.

Materials and methods

We selected 404 patients undergoing consecutive first cycle IVF treatments at our centre between 31 December 2002 and 6 April 2004. First cycle IVF was defined as patients undergoing their first ever attempt at IVF or patients who were undergoing their first cycle of IVF following a clinical pregnancy. Standard IVF with embryo transfer and cycles using ICSI with embryo transfer were included; cycles using donor oocytes were excluded.

Smoking status

Smoking status was obtained from detailed questionnaires completed by each couple on their initial visit to our centre. Current smokers were asked to specify the quantity of cigarettes that they smoked per day, and former smokers were asked the date that they had quit. These questionnaires also contained information about the quantity of alcohol and caffeine imbibed. The height and weight of each patient was used to calculate BMI. Information supplied by the patients was verified by the physician's record whenever possible; no discrepancies between the office chart and the questionnaires were noted.

IVF protocol

Female patients began pituitary down-regulation with a GnRH analogue leuprolide acetate (Lupron, TAP Pharmaceuticals, Lake Forest, IL, USA), 1 mg (20 IU) either on day 21 of the previous menstrual cycle or on day 1 of the treatment cycle ('flare' protocol). Patients began injectible FSH (Follistim, Organon, Roseland, NJ, USA; Gonal F, Serono, Rockland, MA, USA; Bravelle, Ferring Pharmaceuticals, Suffern, NY, USA) on day 3 of the treatment cycle. Dosage of FSH was adjusted based on patients' follicle size and E_2 level; ovulation was then triggered with 10 000 IU of HCG. Oocytes were aspirated transvaginally under ultrasound guidance 36 h after HCG injection. Insemination was performed 4–6 h after oocyte retrieval using either ICSI or mixing oocytes (≤10), with 135 000 spermatozoa per millilitre of insemination media. Patients began vaginal progesterone

supplementation on the day of oocyte retrieval, and embryo transfer was performed 3 days later. The number of embryos to be transferred was decided by the patient in consultation with her physician. Assisted hatching was rarely used.

Outcome measures

Outcome measures and potential confounding variables were obtained from our IVF database containing detailed clinical characteristics and outcomes for every patient undergoing IVF at our centre. Each patient had an FSH level drawn on day 3 of their menstrual cycle before starting IVF. Peak E_2 level and ovarian volume on ultrasound was measured on the day of HCG injection. The number of oocytes retrieved per patient was recorded, and fertilization rate was determined from the proportion of normally fertilized embryos ascertained by the presence of two pronuclei (PN) on the day following insemination. The percentage fertilized was calculated as the number of 2 PN zygotes appearing 18–20 h after insemination divided by the number of oocytes inseminated. The number of oocytes inseminated for IVF was the total number of oocytes available but for ICSI was the total number of mature oocytes available for injection. Embryo quality was based on our embryo score, which considers number of blastomeres, degree of fragmentation and blastomere symmetry. Implantation rate was defined as the number of gestational sacs present on 4-week ultrasound divided by the number of embryos transferred. Positive pregnancy outcome was defined as the presence of fetal heart tones on ultrasound. Live birth outcome included all pregnancies with at least one live birth.

Statistical analysis

Sample size calculation was based on preliminary data, indicating a 22% smoking rate in our <35-year-old population and a 30% rate in our ≥35-year-old population. Based on a pregnancy rate of 65% in our <35-year-old population and 30% in our ≥35-year-old population, the sample size necessary to detect a 25% effect of smoking on pregnancy outcome was 100 per age group assuming $\alpha = 0.05$ and $\beta = 80\%$. This sample size was then doubled to allow for detection of an interaction effect between smoking status and age.

Categorical variables were compared using a χ^2 test. Fisher's exact tests were used in an isolated number of comparisons due to small cell sizes. *P* values estimated from the Fisher's exact tests are summarized in Table I. Continuous variables (BMI, number of oocytes, etc.) were compared using PROC analysis of variance (ANOVA). Relative risks for the association of positive pregnancy and live birth were estimated using a log-binomial regression model estimated using PROC GENMOD. This approach was used because the prevalence of both outcomes exceeded 10%, and logistic regression may have overestimated the size of the effect (McNutt *et al.*, 2003). All analyses were conducted using SAS (version 8.2; SAS Institute, Cary, NC, USA).

Results

Data were missing for 15 patients, leaving 389 patients in the final analysis. Of the 389 women, 36 (9.3%) reported that they were current smokers and 47 (12.1%) reported that they had smoked in the past. Among current smokers, the number of cigarettes per day ranged from <1 to 30. Two-thirds of women (21/30) smoked 10 cigarettes (1/2 a pack) or fewer per day (information not available for six women). Among past smokers, the median number of years since having quit was 4 years with a range of 2 months to 13 years (information not available for three women). A higher percentage of smokers underwent ICSI with a 28.5% smoking incidence in 161 ICSI cycles, versus a 14.8%

Table I. Descriptive characteristics by smoking status

	Never smoked (%)	Currently smokes (%)	Smoked in past (%)	P value
Age				
<35 years	159 (52)	20 (56)	15 (32)	0.03
35+ years	147 (48)	16 (44)	32 (68)	
Partner smoking history				
Never smoked	214 (71)	17 (47)	24 (51)	<0.001
Current smoker	33 (11)	14 (39)	6 (13)	
Past smoker	24 (8)	1 (3)	9 (19)	
Missing or donor sperm	32 (11)	4 (11)	8 (17)	
Gravidity				
0	146 (50)	16 (44)	14 (31)	0.09
1	79 (26)	11 (31)	14 (31)	
2–3	59 (20)	6 (17)	16 (36)	
4+	9 (3)	3 (8)	1 (2)	
Parity				
0	210 (72)	25 (69)	27 (60)	0.36
1	64 (22)	8 (22)	12 (27)	
2+	18 (6)	3 (8)	6 (13)	
Day 3 FSH ≥8	60 (20)	9 (25)	14 (30)	0.24
Day 3 FSH ≥10	25 (8)	5 (14)	3 (6)	0.39
Unexplained infertility	99 (32)	11 (31)	20 (43)	0.36
BMI (mean (SD; kg/m^2)	25.9 (6.5)	24.2 (5.8)	26.4 (7.0)	0.26

smoking incidence in 196 IVF cycles. Thirty-two cycles used both IVF and ICSI, with a 25% smoking incidence in this group.

Current smoking was more common among women <35 years old (56% versus 44%) while a history of smoking was more common among women 35 years and older (68% versus 32%) (Table I). Partner smoking status was associated with patient smoking status ($P < 0.001$). The number of embryos transferred was similar among groups (2.31 ± 0.89 in never smokers, 2.47 ± 1.00 in current smokers and 2.23 ± 0.73 among past smokers). There were non-significant differences in BMI of almost 2 kg/m^2 between current smokers compared with never smokers or former smokers.

The overall relative risk of pregnancy for our entire study group was similar among never smokers, past smokers and current smokers (Table II). The clinical pregnancy rate was 46.4% in IVF cycles and 39.8% in ICSI cycles, and there were no significant differences in pregnancy rates among never, past and current smokers in either of these groups. The clinical pregnancy rate of the nine patients who smoked >10 cigarettes

per day was 55%. The implantation rate was 27.6% for never smokers, 32.6% for current smokers and 27.6% for former smokers. Because the deleterious effects of smoking were expected to become apparent with advancing age, we evaluated the relative risk of pregnancy among women <35, ≥35 and ≥40 years. Risk of pregnancy declined with advancing age; however, smoking status had no significant impact on pregnancy outcome in any age group. The relative risk of pregnancy was also assessed when adjusting for day 3 FSH alone and adjusting for FSH, number of oocytes retrieved and embryo quality score. Smoking status continued to have no significant impact on pregnancy outcome when controlling for these factors. Alcohol consumption and caffeine intake were also evaluated and had no impact on pregnancy outcome.

We then evaluated two age groups (<35 and ≥35 years old), with respect to several more proximate fertility outcomes because pregnancy itself is influenced by multiple variables (Table III). There were no statistically significant differences in peak E_2 levels, log mean ovarian volume, number of oocytes retrieved, oocyte maturity in ICSI, fertilization rate, cleavage rate, embryo quality, percentage of high-quality embryos, pregnancy and live birth among current smokers, former smokers or non-smokers. Smoking status continued to have no significant impact on any of these outcomes when controlling for FSH, number of oocytes retrieved and embryo quality score. There was a trend towards decreasing numbers of high-quality embryos with increasing smoking status, with current smokers having the fewest high-quality embryos in both age groups; however, this trend was not statistically significant. There were also fewer oocytes retrieved and decreased E_2 levels with increasing smoking status in the ≥35-year age group; however, this trend was also not statistically significant.

Discussion

A significant association between smoking and infertility has been purported by many authors, physicians and organizations, although there is no consensus on the magnitude or mechanism of this effect. In our population, 21.4% of IVF patients had past or present exposure to cigarette smoking with no significant effect on pregnancy outcome or on any

Table II. Overall relative risks of pregnancy according to smoking status

Age	Smoking status	Total n	Risk (preg/n)	Relative risk (crude; 95% CI)	Relative risk (adjusted for Day 3 FSH; 95% CI)	Relative risk (adjusted for FSH, number of oocytes retrieved, embryo quality score; 95% CI)
All ages	Never smoked	306	0.43	–	–	–
	Past smoker	47	0.45	1.04 (0.73–1.46)	1.04 (0.74–1.46)	0.99 (0.72–1.37)
	Current smoker	36	0.50	1.15 (0.82–1.65)	1.16 (0.82–1.65)	1.16 (0.85–1.58)
Under 35 years	Never smoked	159	0.53	–	–	–
	Past smoker	15	0.53	1.01 (0.61–1.66)	1.01 (0.61–1.65)	0.88 (0.55–1.40)
	Current smoker	20	0.55	1.04 (0.68–1.59)	1.04 (0.68–1.58)	0.99 (0.66–1.49)
35 years and older	Never smoked	147	0.33	–	–	–
	Past smoker	32	0.41	1.24 (0.77–2.01)	1.25 (0.77–2.02)	1.26 (0.79–2.00)
	Current smoker	16	0.44	1.34 (0.73–2.45)	1.35 (0.74–2.46)	1.50 (0.86–2.61)
40 years and older	Never smoked	45	0.24	–	–	–
	Past smoker	11	0.27	1.12 (0.37–3.33)	1.27 (0.42–3.86)	[a]
	Current smoker	5	0.40	1.17 (0.33–4.20)	1.14 (0.33–4.01)	[a]

[a]Too few events to estimate reliably.

Table III. Pregnancy outcome and proximate fertility outcomes by smoking status in all women and in women <35 years versus women 35 years

	Smoking status	All women	Women <35 years	Women 35 years and older
Number of oocytes (mean ± SD)	Never	12.4 (8.3)	13.8 (9.1)	11.0 (7.2)
	Past	11.4 (7.0)	14.3 (6.1)	10.0 (7.0)
	Current	12.6 (7.3)	16.3 (7.4)	7.9 (3.8)
Percentage fertilization (mean ± SD)	Never	64.9 (23.5)	65.1 (23.0)	64.7 (24.1)
	Past	60.8 (23.9)	64.3 (16.5)	59.1 (26.7)
	Current	64.6 (21.0)	65.1 (20.7)	64.0 (22.0)
Percentage cleaved (mean ± SD)	Never	67.1 (24.7)	67.9 (22.3)	66.3 (25.2)
	Past	66.8 (20.7)	70.2 (13.7)	65.1 (23.3)
	Current	64.9 (23.6)	62.9 (22.9)	67.3 (24.9)
Percentage high-quality embryos (mean ± SD)	Never	40.2 (24.9)	40.3 (23.6)	40.0 (26.7)
	Past	37.3 (20.6)	36.2 (18.7)	38.1 (22.4)
	Current	32.6 (26.6)	32.1 (25.7)	33.6 (29.8)
Embryo score (mean ± SD)	Never	6.80 (1.3)	6.8 (1.3)	6.8 (1.2)
	Past	6.95 (1.1)	7.2 (0.9)	6.8 (1.2)
	Current	6.38 (1.4)	6.5 (1.6)	6.2 (1.2)
Percentage of mature oocytes (mean ± SD)	Never	82.0 (16.2)	81.8 (16.7)	82.3 (15.9)
	Past	76.8 (13.8)	80.3 (11.4)	75.0 (14.9)
	Current	85.8 (12.7)	84.6 (11.1)	87.3 (15.2)
Peak E_2 (pg/ml)	Never	1867 (939)	1974 (977)	1754 (886)
	Past	1608 (875)	1695 (702)	1566 (956)
	Current	1782 (853)	2216 (809)	1267 (583)

All P values >0.05.

other more proximate measures of fertility. Studying the effect of smoking on IVF outcome is fundamentally difficult because of multiple confounding variables, including certain factors associated with smoking that may themselves contribute to subfecundity. We controlled for day 3 FSH, age, BMI, embryo quality, number of oocytes retrieved, caffeine and alcohol use in our large cohort and failed to demonstrate even a small effect of smoking on IVF outcome. However, our study did demonstrate that a higher proportion of smokers in our population underwent ICSI, which is likely a reflection of a higher incidence of male factor infertility among male smokers, because female smoking and male smoking were positively correlated in our population.

We slightly underestimated the smoking rate, which was 21% in our final analysis, whereas our original power analysis assumed a 22% smoking rate in our <35-year-old population and a 30% rate in our ≥35-year-old population. However, our sample size was nearly twice the number of women needed to have 80% power to detect a 25% effect of smoking on pregnancy outcome. Retrospective analysis reveals that we were powered to detect a 15–20% effect of smoking on pregnancy outcome. A larger sample size would be necessary to detect a <15% difference in pregnancy outcome between smokers and non-smokers.

Some literature supports a dose-dependent effect of smoking on fertility, and the majority of our current smokers smoked ≤10 cigarettes a day. Although the clinical pregnancy rates of women who smoked >10 cigarettes per day in our population were not significantly different from non-smokers, our limited sample size of heavy smokers makes this finding difficult to interpret. Studying a population with a larger proportion of heavy smokers could yield a different result. Our results also relied on patient reporting of smoking status and it is possible, given the publicity about smoking and infertility, that some of our patients failed to completely disclose their smoking habits. Measuring cotinine levels is a potentially more reliable method

of determining smoking status; however, it does not allow for detection of former smokers, which constitute a significant proportion of women undergoing fertility treatments. Another recent study evaluating the effect of smoking on IVF outcome did measure cotinine levels and also found no effect of recent or ongoing smoking on pregnancy rates (Ellenbogen, 2004).

That several large studies, including ours, fail to show a detrimental effect of smoking on IVF outcomes raises interesting questions regarding the effects of controlled ovarian stimulation and selection of high-quality embryos on pregnancy rates in smokers. Although the primordial and early-growing follicles, the primary contributors to ovarian reserve, are almost completely devoid of blood supply, several studies have shown that environmental contaminants reach the ovary and have been quantified in follicular fluid (Trapp *et al.*, 1984; Zenzes *et al.*, 1996; Campagna *et al.*, 2001; Younglai *et al.*, 2002). This suggests that toxins would have an effect on oocyte quality that is unlikely to be overcome by ovarian stimulation. However, assisted reproduction technology (ART) allows the opportunity to select high-quality embryos, which may explain why the pregnancy rates of smokers and non-smokers are similar. Embryo quality score has been proposed as a better predictor of pregnancy than female age or number of transferred embryos (Terriou *et al.*, 2001). Our data confirm that embryo quality score was positively associated with pregnancy outcome (Pearson's correlation 0.25, $P < 0.001$). Although there were no statistically significant differences in our population with respect to percentage high-quality embryos by smoking status, there was a trend in our data towards increasing embryo quality with decreasing smoking status. This may indicate decreased ovarian reserve in smokers.

The question of whether smoking affects IVF outcome is significant to patient care given that some insurance companies deny coverage for IVF to women who smoke. Cigarette smoking is associated with multiple medical problems, including an increased risk of cardiovascular disease, lung cancer, cervical

cancer, emphysema and cerebrovascular disease (Watson and Conte, 1954; Franklin *et al*., 1956; Jenkins *et al*., 1968; Kannel *et al*., 1976; Clarke *et al*., 1982; Seltzer, 2003). Moreover, smoking during pregnancy carries an increased risk of deleterious effects on both mother and fetus, including spontaneous miscarriage, intrauterine growth restriction, placental abruption and stillbirth (Centers for Disease Control and Prevention, 2004; Wainright, 1983; Hammoud *et al*., 2005; Kyrklund-Blomberg *et al*., 2005). Thus, there is ample reason for physicians to counsel their patients to stop smoking. However, on the basis of our findings, we should be cautious about attributing a direct effect of smoking on fertility treatment outcomes.

Acknowledgements

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