Human Reproduction Vol.16, No.7 pp. 1382–1390, 2001

Effects of female and male smoking on success rates of IVF and gamete intra-Fallopian transfer

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BACKGROUND: Smoking by both male and female partners may play a significant role in the success rates of assisted reproductive technologies. The objective of this 5-year prospective study was to investigate the influence of cigarette smoking by the wife, husband, and couple at various time points (e.g. lifetime, week prior, or during the procedures) on different biological parameters of IVF and gamete intra-Fallopian transfer (GIFT). METHODS AND RESULTS: A total of 221 couples, aged >20 years, of Caucasian, Black, Asian or Hispanic descent were recruited from seven infertility clinics located in Southern California. Couples (i.e. either female or male or both) who ever smoked compared with non-smokers, had adjusted relative risks (RR) of 2.41 (95% CI 1.07–5.45, $P = 0.03$) of not achieving a pregnancy, and 3.76 (95% CI 1.40–10.03, $P < 0.01$) of not having a live birth delivery, while adjusting for potential confounders. For couples who smoked for >5 years, there was an adjusted RR = 4.27 of not achieving a pregnancy (95% CI l.53–11.97, $P = 0.01$). The number of oocytes retrieved decreased by 40% for couples (smokers, $n = 6$) and by 46% for men who smoked during the week of the visit for IVF or GIFT. Women who smoked in their lifetime had adjusted risks of 2.71 of not achieving a pregnancy (95% CI 1.37–5.35, $P < 0.01$), and 2.51 (95% CI 1.11–5.67, $P < 0.03$) of not having a live birth delivery. CONCLUSIONS: There is compelling evidence that couples should be made aware that smoking years before undergoing IVF and GIFT can impact treatment outcome. This study may also provide insight into the timing and effects of male and female smoking on natural reproduction.

Key words: assisted reproductive technologies/IVF/lifestyle habits/success rates/tobacco smoke

Introduction

In the United States, 2.7 million couples of childbearing age are infertile (Session *et al.*, 1998). By the year 2025, between 5.4 and 7.7 million women will be diagnosed with infertility (Grainger and Tjaden, 2000). The dollar value of personal, familial and societal losses, coupled with the emotional ramifications of infertility are inestimable. There is a subgroup of infertile couples who have exhausted all forms of conventional therapy for infertility.

IVF and gamete intra-Fallopian transfer (GIFT) procedures resulted in ~26% and 29% delivery rates per retrieval (American Society for Reproductive Medicine/Society for Assisted Reproductive Technology, 1999) respectively, compared with natural reproduction, which has a mean success rate of 25% per cycle (Taymor, 1990). Lifestyle habits such as smoking may play a pivotal role in the success rates of IVF and GIFT. Tobacco smoke contains several hundred substances including nicotine, carbon monoxide and mutagens (e.g. radioactive polonine, benzo[a]pyrene, naphthalene and methylnaphthalene) (Stillman *et al.*, 1986).

There are currently 10 retrospective studies (Elenbogen *et al.*, 1991; Pattison *et al.*, 1991; Rosevear *et al.*, 1992; Shahara *et al.*, 1994; Maximovich and Beyler, 1995; Sterzik *et al.*, 1996; Van Voorhis *et al.*, 1996; El Nemr *et al.*, 1998; Joesbury *et al.*, 1998; Weigert *et al.*, 1999), three prospective studies (Trapp *et al*., 1986; Harrison *et al*., 1990; Hughes and Brenna, 1996), and three meta-analyses (Hughes *et al.*, 1992; Feichtinger *et al.*, 1997; Augood *et al.*, 1998) which have investigated the effect of smoking on IVF and GIFT.

In all of these studies, smoking did not uniformly affect the same endpoints. Maternal smoking resulted in decreased fertilization rates (Elenbogen *et al.*, 1991; Rosevear *et al.*, 1992; Shahara *et al.*, 1994), decreased numbers of oocytes (Harrison *et al.*, 1990), decreased pregnancy rates (Harrison *et al.*, 1990; Elenbogen *et al.*, 1991; Rosevear *et al.*, 1992; Maximovich and Beyler, 1995; Van Voorhis *et al.*, 1996; Feichtinger *et al.*, 1997; Augood *et al.*, 1998; Joesbury *et al.*, 1998) and increased miscarriage rates (Pattison *et al.*, 1991). In contrast, in other studies there was no effect of smoking on fertilization (Trapp *et al.*, 1986; Pattison *et al.*, 1991; Weigert

et al., 1999) and pregnancy rates (Trapp *et al.*, 1986; Hughes and Brenna, 1996; Sterik *et al.*, 1996; El Nemr *et al.*, 1998; Weigert *et al.*, 1999). Many studies did not have adequate power to assess significant differences in pregnancy outcomes (Trapp *et al.*, 1986; Elenbogen *et al.*, 1991; Shahara *et al.*, 1994). Other methodological limitations included not accounting for any potential confounders such as age, race, socioeconomic status, occupation, reproductive characteristics (e.g. parity, type of infertility, number of attempts) and other lifestyle habits (e.g. medication, recreational drug use, alcohol consumption) (Seibel, 1980; Trapp *et al.*, 1986; Weiss and Eckert, 1989; Harrison *et al.*, 1990; Elenbogen *et al.*, 1991; Shahara *et al.*, 1994). The definition of smoking history in these studies was not of sufficient detail, failing to differentiate the quantity, type (cigarette, cigar, chewing tobacco) and timing of smoking (i.e. past smoking history versus during the IVF procedure). Smoking was classified when patients first entered the study, but not throughout the procedure when habits dramatically changed (Seibel, 1980). In addition, women who quit smoking cigarettes after entry into the study would be misclassified as smokers. Finally, the contribution of the male partner's smoking history, although covered in two studies (Hughes and Brenna, 1996; Joesbury *et al.*, 1998), was entirely omitted in the majority of studies (Trapp *et al.*, 1986; Weiss and Eckert, 1989; Harrison *et al.*, 1990; Elenbogen *et al.*, 1991; Rosevear *et al.*, 1992).

The current study evaluated all biological and reproductive endpoints (including pregnancy outcomes) of IVF and GIFT, involved both females and males, performed multivariate analyses and adjusted for potential confounders and interaction terms, and prospectively measured smoking habits (i.e. quantity, frequency and duration).

Materials and methods

Hypothesis

Smoking may have an adverse effect on all biological endpoints of IVF and GIFT, including sperm indices, number of oocytes retrieved, fertilization rates, number of embryos transferred, and pregnancy rates, resulting in lower success rates (i.e. live birth delivery rates).

Study design

A prospective (observational) study was conducted to evaluate the effects of lifetime and current smoking by either the female or male partner or couple, on the reproductive endpoints of IVF and GIFT.

Subjects

This study included 221 couples undergoing IVF or GIFT in seven centres in Los Angeles, Orange and San Diego Counties, who were recruited between July 1993 and June 1998. These centres were chosen because of the physicians' expertise and uniformly high success rates with assisted reproductive technologies. In all instances, appropriate conventional forms of therapy for infertility, including surgical or medical therapy for endometriosis, and intrauterine insemination for cervical factors, had failed. Each woman's evaluation consisted of a minimum of laparoscopy, hysteroscopy and hysterosalpingography, while the men underwent a semen analysis and sperm penetration assay. Additional tests offered at the clinics included screening for rubella, HIV, cytomegalovirus, hepatitis B and syphilis, as well as psychological testing.

Inclusion criteria

All couples taking part in IVF or GIFT procedures, were at least 20 years of age, had a stable marital or co-habiting relationship, and were of Caucasian, Black, Hispanic or Asian descent. Couples with primary or secondary infertility were accepted as candidates if their infertility was due to tubal disease, endometriosis, immunological causes or male factor, or was unexplained. In addition, to be eligible, all women had to be diagnosed with female infertility problems, and undergoing only one treatment cycle during the study period. Women aged >35 years were analysed separately because of an increased incidence of miscarriage and a higher risk of chromosomal abnormality (Padilla and Garcia, 1989). The study sample is representative of the age, race and education level of couples enrolling in IVF and GIFT programmes in Southern California.

Exclusion criteria

Women with pre-existing illnesses requiring medical management during pregnancy (i.e. high blood pressure, heart disease, diabetes, thyroid or renal disease) were excluded from the study. Donor spermatozoa and oocytes and surrogate uteri were not included in this study, as the personal lifestyle habits and reproductive/medical history were unknown. Only fresh, non-donor IVF/GIFT cycles were included. Since many couples underwent more than one IVF attempt or cycle (defined from stimulation to embryo transfer), no women who achieved pregnancies in subsequent cycles were included.

IVF and GIFT procedures

The technical aspects of the procedures were standardized across study centres, all of which had similar pregnancy rates in general (historically) following IVF and GIFT; hence, these factors would not confound the relationship between smoking and success rate. Differences between study sites included dosages, the initial drug of choice, and whether human menopausal gonadotrophin or a pure FSH preparation was used. In this study, women not requiring ovarian stimulation were omitted.

Definition of IVF and/or GIFT outcomes

The impact of smoking on IVF and GIFT was measured at various endpoints, including the number of oocytes retrieved (aspirated), sperm profile (e.g. motility, morphology, count), fertilization, embryo transfer, pregnancy and delivery.

The pregnancies in the five centres were categorized according to the following outcomes. Biochemical pregnancy was diagnosed by an elevated serum human chorionic gonadotrophin (HCG) concentration obtained at 16–20 days after oocyte retrieval, followed by decline and disappearance of measurable HCG prior to the gestational sac appearing on ultrasound (Jones *et al.*, 1983; Yuzpe *et al.*, 1983). Clinical pregnancy was confirmed by elevated serum HCG concentration on successive occasions measured every 5–7 days, and ultrasound verification of at least one gestational sac (The American Fertility Society, 1989); ectopic and biochemical pregnancies were excluded from this category. An ectopic pregnancy was defined as a pregnancy outside the uterus (Williams, 1985). Preterm birth referred to a baby born from 32–36 weeks, while a premature birth was a baby born before 32 weeks (Williams, 1985). A term delivery indicated a pregnancy of >37 weeks, but <42 weeks (Williams, 1985). Multiple gestations referred to more than one gestational sac or fetus (Williams, 1985). A low birth weight infant was defined as an infant weighing <2500 g at birth (The American Fertility Society, 1989).

Data collection

Before conducting this research, institutional approval and informed consent was obtained from all couples involved in IVF or GIFT

treatment who wished to participate in the study. The questionnaire ascertained information on demographic characteristics (age, race, socioeconomic status, education, occupation, marital status), and medical and reproductive histories. Clinical information pertained to the assisted reproduction treatment cycle (e.g. dose, type, and duration of hormones, number of oocytes retrieved), as well as information on the pregnancy and birth, and neonatal characteristics. Technical aspects of the procedure (e.g. dose, type, and duration of hormones, number of oocytes retrieved or fertilized, number of embryos transferred, sperm parameters) were obtained primarily from the patient's medical records, though some of this information was also recorded in the questionnaires.

In this study, the predictor variables were grouped into four categories: demographic characteristics of the couple (e.g. age, race, occupation, education); medical and reproductive information (e.g. age at menarche, age at first intercourse, number of sexual partners, history of sexually transmitted diseases, number of years of infertility, parity, and gravidity); procedural issues (indications, number of attempts, hormonal stimulation); and other personal lifestyle habits (e.g. alcohol intake, medication, caffeine, recreational drugs).

Definition of smoking

The smoking habits of couples were ascertained, beginning at baseline, which included the following time periods: lifetime, and 1 year, 1 month, 1 week and 1 day prior to the procedure. In addition, the couples also provided detailed smoking information during the procedure and afterwards, until the end of the pregnancy outcome. A total of five questionnaires was distributed to the mother and father at the initial clinic visit, to the mother during embryo transfer and the father during sperm collection, and to the mother at the pregnancy outcome (i.e. live birth delivery). This protocol was chosen to obtain as valid a smoking history as possible throughout the procedure, without the responses being influenced by the success or failure of the procedure, as well as to prevent influencing smoking habits during IVF or GIFT. The smoking history was buried in the questionnaire among other lifestyle habits in order not to reveal the hypothesis.

A male and female baseline smoking history (lifetime and one year prior) was obtained to determine the average number of cigarettes (or if applicable, cigars or chewing tobacco) smoked per week, the brand used, and the age that the woman or man first started and stopped (if applicable) smoking. A very detailed smoking history for each parent was recorded during the IVF or GIFT procedure, including the brand, number of cigarettes and periods of abstinence one week before, one day before, and during the procedure by each partner; changes in smoking pattern such as from high- to low-yield cigarettes; exposure to passive smoke by the male or female spouse at home, or in the work environment, was also recorded. Any potential dose–response effect linking the number of cigarettes smoked and an adverse outcome (e.g. decreased number of oocytes aspirated) of IVF or GIFT was noted.

Statistical analysis

Descriptive characteristics of the women and men undergoing IVF/GIFT were calculated. For continuous variables, the mean, median, standard deviation and ranges were determined, while proportions were used for categorical variables (Table I).

A preliminary analysis entailed calculation of unadjusted relative risks and 95% confidence intervals (CI) for the effects of smoking at various time periods (e.g. lifetime, year, month, week and day prior to, and during the procedure) on each of the dichotomous biological endpoints of successful pregnancy, live birth delivery and sperm parameters. The unadjusted relative risk (RR) for males, females and couples are presented as a source for comparisons with the other published studies, since very few performed multivariate analyses. A least squares analysis was conducted to determine univariate rates of change of the continuous endpoints: number of oocytes aspirated (retrieved) and fertilized, and embryos transferred. For the subset of women who had successful live birth deliveries, the effects of smoking on birth weight and multiple deliveries were determined. The variables 'number of oocytes aspirated' and 'number of oocytes fertilized' were transformed to a logarithmic scale in order to fulfil the Gaussian assumptions of least squares regression.

Stratified analyses were performed to determine whether an individual risk factor (i.e. number of attempts or age) could explain an observed association between smoking and each endpoint. To evaluate the effect of smoking on each biological endpoint while simultaneously adjusting for all four categories of confounders (e.g. demographic characteristics, reproductive history, other lifestyle habits and procedural differences), logistic regression (Williams, 1985) was conducted for each dichotomous variable (e.g. pregnancy, live birth delivery, multiple births), and linear regression (Kleinbaum *et al.*, 1982) was used for each continuous variable (e.g. number of oocytes retrieved and transferred, number of embryos fertilized, and infant birth weight). Only variables which acted as confounders of the smoking/IVF or GIFT association were retained in the final models. There were many predictors in this study; therefore, interactions between the potential confounders (e.g. age or number of attempts) and smoking variables were assessed (when biologically meaningful) to ascertain whether the effects of smoking on the reproductive endpoints were modified by other risk factors. In particular, stratified analyses were performed and RR between strata were compared. Interaction terms were also included in the multivariate analyses. Analyses were conducted using the statistical software package S Plus (Math Soft Inc., Seattle, WA, USA).

Results

The study included 221 women (2 of whom had their age missing) of mean (± SD) age 36.42 ± 4.26 (range 26 to 49) years. Of these women, 125 (58%) and 51 (23%) were aged >35 and $\geqslant 40$ years respectively. Approximately 76% of women were college-educated, and 85% were employed while undergoing IVF or GIFT. The racial breakdown for women was 75% Caucasian, 14% Asian, 6% Hispanic, 2% African-American and 3% unknown (Table I).

Among the women, 50.2% had never smoked, 47.1% had smoked in their lifetime, and in 2.7% the smoking status was unknown. The mean time period that women smoked was 3.77 ± 6.7 (range 1 to 26) years. A total of 9% of women undergoing IVF or GIFT reported smoking the year before, whereas 7% reported smoking the month before, and $<4\%$ recorded the week or day prior to, or during the procedure.

A number of the men refused to answer questions on lifestyle habits; a total of 55 males had missing information on smoking. Among 166 men of mean age 38.4 ± 5.68 (range 22 to 55) years, ~75% undergoing IVF or GIFT were college-educated, and 93% were employed. The racial composition of the male sample was 80% Caucasian, 11% Asian and 6% Hispanic; the remaining men were either African- or Native-American or unknown. Among men undergoing IVF or GIFT, 42.1% had never smoked, 42.5% had smoked in their lifetime, and in 15.4% the smoking history was unknown. The mean

Table I. Demographic and reproductive characteristics of couples undergoing IVF or GIFT

Characteristic	Maternal characteristics		Paternal characteristics	
	n[a]	%	*n*[a]	%
Age[b]	219	36.42 ± 4.26	166	38.4 ± 5.68
Race				
White	165	74.66	133	80.12
Asian	32	14.48	18	10.84
Hispanic	13	5.88	10	6.02
African-American	4	1.81	2	1.2
Native American			1	0.6
Unknown	2	3.2	2	1.2
Maternal education				
Duration (years)[b]	207	16.81 ± 2.57	154	16.93 ± 2.71
Completed college	167	75.57	125	75.30
Did not complete college	40	18.10	29	17.47
Unknown	14	6.33	12	7.23
Employment				
Yes	188	85.07	155	93.37
No	27	12.22	0	0.0
Unknown	6	2.71	11	6.63
Parity				
0	171	77.38	–	–
1	39	17.65	–	–
2	7	3.17	–	–
3	2	0.90	–	–
Unknown	2	0.90	–	–
Years of infertility[b]	217	4.18 ± 3.05	–	–
Indications				
Tubal	74	33.48	–	–
Endometriosis	48	21.72	–	–
Male	59	26.70	–	–
Idiopathic	44	19.91	–	–
Other	53	23.98	–	–
Type of procedure				
IVF	136	61.54	–	–
GIFT	79	35.75	–	–
ZIFT	6	2.71	–	–
ART attempts[b]	221	1.67 ± 1.12	–	–

[a]Number of subjects on whom complete data were available for each characteristic.
[b]Values are mean ± SD.
ZIFT = zygote intra-Fallopian transfer.

period of smoking by males was 4.2 ± 7.31 (range 1 to 31) years. A total of 7% of males reported smoking the year before, 5% of men the month before, and ⩽6% for the week or day prior, or during the procedure.

For couples [defined as either one (female or male) or both partners], 62% reported ever smoking in their lifetime, 22% never smoked, and for the remaining 16% this information was unknown; 15% of couples smoked the year before, 11% smoked the month before, and <9% the week or day prior to or during the procedure.

In this study, the average number of embryos transferred per patient was 3.95 (median = 4; range 1–8). In total, 60% of women were on their first attempt, and totals of 16, 10 and 9% respectively corresponded to the second, third and fourth or more attempt (in 5% the information was missing). The overall average success rates for pregnancy and live-birth delivery were 32% (n = 71) and 19% (n = 41) respectively. The pregnancy rates for the different study sites ranged from 18 to 47%, while live birth delivery rates ranged from 11 to 30%.

Smoking couples and IVF and GIFT outcomes

Univariate analyses

A smoking couple was defined as either one or both partners smoking at a specified time period (e.g. lifetime, one year, month, week or day before, or during the visit for IVF or GIFT). When conducting univariate analyses, among those couples who smoked and underwent IVF or GIFT, the RR of not getting pregnant was twice (RR = 2.0, 95% CI 1.01–3.96, $P < 0.05$) that compared with couples who never smoked (Table II). For each additional year that the couple smoked, the risk of not getting pregnant increased by 4%. Among those IVF or GIFT couples who smoked for >5 years, the risk of not getting pregnant from IVF or GIFT was 2.96 (95% CI 1.30–6.74, $P = 0.01$) compared with those who never smoked (Table III).

There was a 45% decrease (log coefficient = –0.62, $P = 0.01$) in the number of oocytes aspirated for smokers during the week of the IVF or GIFT procedure (Table IV). Among smoking couples compared with non-smokers, the RR

Table II. The effect of ever smoking during their lifetime on the biological endpoints of IVF or GIFT procedures[a]

Univariate analyses *Reproductive outcome*	n[b]	Relative risk	95% CI	P
Couple smoking				
Never pregnant	178	2.00	[1.01, 3.96]	<0.05
Live delivery	170	2.48	[1.14, 5.38]	0.02
Wife smoking				
Never pregnant	206	2.50	[1.38, 4.55]	<0.01
Live delivery	197	2.25	[1.10, 4.58]	0.03
Multivariate analyses				
Reproductive outcome	n	Adjusted relative risk[c]	95% CI	P
Couple smoking				
Never pregnant	171	2.41	[1.07, 5.45]	0.03
Live delivery	164	3.76	[1.40, 10.03]	<0.01
Wife smoking				
Never pregnant	198	2.71	[1.37, 5.35]	<0.01
Live delivery	190	2.51	[1.11, 5.67]	0.03

[a]Only statistically significant results are presented in this table.
[b]Number of subjects on whom complete data were available for each characteristic.
[c]Adjusted for female age, female education, female race, parity, type of procedure, number of attempts, female alcohol, marijuana and recreational drug use over various time periods.

Table III. The effects of the duration of smoking (number of years) on the biological endpoints of IVF or GIFT procedures[a]

Univariate analyses

Reproductive outcome	n[b]	Effect	Relative risk	95% CI	P
Couple smoking					
Never pregnant	172	No. of years smoked	1.04	[1.003, 1.07]	0.03
Never pregnant	172	Smoked >5 years	2.96	[1.30, 6.74]	0.01
Live delivery	164	Smoked >5 years	3.00	[1.14, 7.86]	0.03
Wife smoking					
Never pregnant	202	No. of years smoked	1.09	[1.02, 1.15]	<0.01
Never pregnant	206	Smoked >5 years	4.32	[1.78, 10.48]	<0.01
Live delivery	193	Smoked >5 years	2.92	[1.04, 8.19]	0.04

Multivariate analyses

Reproductive outcome	n[b]	Effect	Adjusted relative risk[c]	95% CI	P
Couple smoking					
Never pregnant	164	Smoked >5 years	4.27	[1.53, 11.97]	0.01
Wife smoking					
Never pregnant	193	No. of years smoked	1.09	[1.01, 1.16]	0.02
Never pregnant	193	Smoked >5 years	4.86	[1.77, 13.29]	<0.01

[a]Only statistically significant results are presented in this table.
[b]Number of subjects on whom complete data were available for each characteristic.
[c]Adjusted for female age, female education, female race, parity, type of procedure, number of attempts, female alcohol, marijuana and recreational drug use over various time periods.

of no live birth delivery with IVF or GIFT was 2.48 (95% CI 1.14–5.38, $P = 0.02$) (Table II). Among couples who smoked for >5 years compared with non-smokers, the RR was 3.0 (95% CI 1.14–7.86, $P < 0.03$) of no live birth delivery (Table III).

There was no dose–response effect for the number of cigarettes smoked during any time period (e.g. lifetime, during the clinic visit) and any of the reproductive outcomes (e.g. fertilization, pregnancy), possibly because the number of reported smokers was very small.

Table IV. The effects of smoking period (i.e. week before or during procedure) on the biological endpoints of IVF or GIFT[a]

Univariate analyses

Reproductive outcome	n[b]	Smoking period	Coefficient (log)	Change (%)	95% CI	P
Couple smoking						
Oocytes aspirated (log)	133	Week of procedure	–0.62	–45	[–1.10, –0.14]	0.01
Husband smoking						
Oocytes aspirated (log)	139	Week before procedure	–0.51	–40	[–0.93, –0.09]	0.02
Oocytes aspirated (log)	140	Week of procedure	–0.68	–49	[–1.16, –0.19]	<0.01

Multivariate analyses

Reproductive outcome	n[b]	Smoking period	Coefficient (log)	Change (%)	95% CI	P
Couple smoking						
Oocytes aspirated (log)	126	Week of procedure	–0.52	–40[c]	[–1.02, –0.004]	<0.05
Husband smoking						
Oocytes aspirated (log)	133	Week before procedure	–0.55	–42[d]	[–1.01, –0.10]	0.02
Oocytes aspirated (log)	130	Week of procedure	–0.61	–46[d]	[–1.11, –0.11]	0.02

[a]Only statistically significant results are presented in this table.
[b]Number of subjects on whom complete data were available for each characteristic.
[c]Adjusted for female age, female education, female race, parity, type of procedure, number of attempts, female alcohol, marijuana and recreational drug use over various time periods.
[d]Adjusted for female age, female education, parity, type of procedure, number of attempts, male alcohol, marijuana and recreation drug use over various time periods.

Multivariate analyses

When conducting multivariate analyses, the RR of not achieving a pregnancy was 2.41 (95% CI 1.07–5.45, $P = 0.03$) for smoking compared with non-smoking couples undergoing IVF or GIFT, while adjusting for the woman's age, race and education, type of procedure, number of attempts, parity, and maternal alcohol, marijuana and recreational drug consumption (Table II). The RR of not becoming pregnant was 4.27 (95% CI 1.53–11.97, $P = 0.01$) for couples who smoked for >5 years compared with non-smoking couples, while adjusting for the above-mentioned covariates (Table III). The RR of not achieving a live birth delivery among those couples who smoked compared with non-smokers undergoing IVF or GIFT was 3.76 (95% CI 1.40–10.03, $P < 0.01$) (Table II). There was a 40% decrease (log coefficient = –0.52, $P < 0.05$) in the number of oocytes aspirated from smoking couples during the visit for IVF or GIFT, though only six couples smoked during this time period (Table IV).

There were 41 couples who had successful live birth deliveries. Of these couples, 11 had multiple births (nine delivered twins, and two delivered triplets). The effect of smoking on multiple births was assessed using logistic regression. The risk of multiple deliveries was 9% higher (RR = 1.09, $P < 0.05$) for each additional year that couples smoked before undergoing IVF or GIFT, while adjusting for the woman's age, attempt, and number of embryos transferred. When the tubal factor was added to the model, the RR changed marginally (to 1.11, $P = 0.04$).

Statistically significant interactions between the number of attempts and smoking, and maternal age and smoking were also tested. When multivariate analyses were performed without adjustment for drugs or marijuana, and attempt number was categorized into two groups (e.g. one versus more than one attempt), all RR ratios were marginally lower, decreasing by at most 0.5, although significance values remained the same.

Analysis was also conducted while stratifying on maternal age (⩽35 years versus >35 years). The results across groups were fairly consistent, although the risks were higher in the older age group. However, the significance values were higher, possibly because of the smaller numbers in each stratum.

Female smoking and IVF and GIFT outcomes

Univariate analyses

If a woman ever smoked during her lifetime, this more than doubled her risk of not achieving a pregnancy (RR = 2.50, 95% CI 1.38–4.55, $P < 0.01$) (Table II); moreover, each year that she smoked increased the odds of not becoming pregnant by 9% (95% CI 1.02–1.15, $P < 0.01$) (Table III). The risk of not becoming pregnant among women who smoked for >5 years was 4.32 (95% CI 1.78–10.48, $P < 0.01$) compared with those women who never smoked while undergoing IVF or GIFT (Table III). If a woman ever smoked in her lifetime, she

had 2.25 times (95% CI 1.10–4.58, $P = 0.03$) the RR of not having a healthy live birth, compared with a non-smoker undergoing IVF or GIFT (Table II); if she smoked for >5 years, the RR of not giving birth to a healthy baby was 2.92 (95% CI 1.04–8.19, $P = 0.04$) (Table III).

Multivariate analyses

If a woman ever smoked in her lifetime, the risk of not achieving a pregnancy while undergoing IVF or GIFT was 2.71 (95% CI 1.37–5.35, $P < 0.01$) compared with a non-smoker, while adjusting for maternal age, race and education, type of procedure, number of attempts, parity, and maternal alcohol, marijuana and recreational drug consumption (Table II). Each additional year that she smoked increased the risk of no pregnancy by 9% (RR = 1.09, 95% CI 1.01–1.16, $P = 0.02$) (Table III), while for those women who smoked for >5 years this risk was 4.86 (95% CI 1.77–13.29, $P < 0.01$) compared with non-smokers (Table III). Women who ever smoked ultimately had a RR of 2.51 (95% CI 1.11–5.67, $P = 0.03$) of no live birth delivery with either IVF or GIFT (Table II).

The multivariate analyses were repeated without recreational drug or marijuana use in the models, and there were no significant changes in the results. Interactions between maternal age and maternal smoking were not statistically significant; stratifying by age (≤35 years versus >35 years) did not substantially alter the results.

Male smoking and IVF and GIFT outcomes

Univariate analyses

For men who smoked one week before the IVF/GIFT visit, there was a 40% decrease (log coefficient = –0.51, $P = 0.02$) in the number of oocytes aspirated compared with non-smokers, whereas if they smoked during the week of the procedure there was a 49% decrease (log coefficient = –0.68, $P < 0.01$) in the number aspirated (Table IV). Men who smoked for >5 years had a 7.55-fold higher risk for multiple births compared with non-smokers (95% CI 1.09–51.87, $P = 0.04$).

Multivariate analyses

If men smoked the week before the procedure, there was a 42% decrease (log coefficient = –0.55, $P = 0.02$) in the number of oocytes aspirated, while adjusting for maternal age, maternal education, type of procedure, number of attempts, parity and female alcohol consumption (Table IV). Furthermore, if men smoked during the week of IVF or GIFT, there was a 46% decrease (log coefficient = –0.61, $P = 0.02$) in the number of oocytes aspirated, while adjusting for the above-mentioned co-variates (Table IV). For men who smoked during their lifetime, there was a 5.42 higher risk (95% CI 1.01–29.19, $P < 0.05$) of multiple births during IVF or GIFT, while adjusting for the woman's age, attempt number and number of embryos transferred. For those men who smoked for >5 years, there was 9.04-fold higher risk of delivering multiple births during the IVF or GIFT cycle (95% CI 1.17–69.65, $P = 0.04$).

Men who smoked for either ≤5 years or >5 years produced babies who weighed 20 and 21% less respectively than the babies produced by non-smokers, while adjusting for age and number of attempts. When only the subset of single deliveries was analysed, men who smoked for ≤5 years produced babies weighing 14% less than the infants produced by non-smokers ($P = 0.05$), while adjusting for maternal age and number of attempts. Because there were very few men who smoked for >5 years within the single live birth group, there was possibly insufficient power to detect any effect of their smoking behaviour on birth weight.

Discussion

The current study provides compelling evidence that smoking by both couples and women, at any time during their lifetime, or for a period of >5 years, negatively affects the chances of achieving a pregnancy and pregnancy outcome (i.e. live birth delivery). For both couples and women smoking during their lifetime, the adjusted RR of not achieving a pregnancy were 2.4 and 2.7 respectively while adjusting for the woman's age, race and education, parity, type of procedure, number of IVF or GIFT attempts, and female alcohol, recreational drug and marijuana use. If the couple or woman smoked for >5 years, this risk increased to 4.27 and 4.86 respectively while adjusting for the above-mentioned potential confounders.

The deleterious effect of smoking also became detectable in older women undergoing IVF treatment (Zenzes and Reed, 1997). In the current study, in the older age group (>35 years), the risk of not achieving a pregnancy was 4.64 ($P = 0.01$) for those couples who smoked for >5 years compared with non-smokers undergoing IVF or GIFT.

Six studies (Harrison *et al.*, 1990; Elenbogen *et al.*, 1991; Pattinson *et al.*, 1991; Rosevar *et al.*, 1992; Maximovich and Beyler, 1995; Van Voorhis *et al.*, 1996) confirmed our findings of a lower number of pregnancies among women who smoked, while five studies (Trapp *et al.*, 1986; Hughes and Brenna, 1996; Sterzik *et al.*, 1996; El Nemr *et al.*, 1998; Weigert *et al.*, 1999) reported no statistically significant differences between smokers and non-smokers; one study (Joesbury *et al.*, 1998) reported that male smoking had a deleterious effect on pregnancy outcome (12-week pregnancy).

A meta-analysis (Feichtinger *et al.*, 1997) (including seven datasets and the authors' own data) revealed that the pregnancy rates among women non-smokers (21%) were significantly higher than smokers (14%) after their first attempt at IVF, and almost twice as many IVF cycles were needed for smokers as compared with non-smokers to become pregnant [odds ratio (OR) =1.79, 95% CI 1.24–2.59]. Another group (Augood *et al.*, 1998) also performed a meta-analysis of nine studies, and calculated an OR of 0.66 for pregnancy per number of IVF treated cycles in smokers versus non-smokers. All of these studies, apart from two (Hughes and Brenna, 1996; Joesbury *et al.*, 1998) did not evaluate men or couples, and did not adjust for potential confounders.

Studies of females undergoing IVF are also contradictory with regards to fertility rates and numbers of retrieved oocytes (Zenzes and Reed, 1997). In women, it has been reported that smoking alters the meiotic spindle of oocytes, leading to chromosomal errors which affect reproductive outcomes

(Zenzes, 2000). Hence, smoking was associated with reduced numbers of retrieved oocytes (Zenzes *et al*., 1995, 1998; Zenzes and Reed, 1997). Furthermore, in mice, nicotine disrupted the rate of oocyte maturation, reduced ovulation and fertilization rates, and increased diploidy (Mailhes *et al*., 2000). The current study showed no effect of female smoking on oocyte retrieval; however, for men and couples who smoked during the week prior to, or during the procedure, the number of oocytes retrieved decreased by 42 and 46% respectively, possibly because of the effects of passive smoking on the women. The biological plausibility for this result is difficult to explain, and the reasons why no significant decrease was detected in the number of retrieved oocytes from female smoking, despite a very high decrease from male smoking, can only be speculated upon. This finding might be attributed to there being a lack of power to detect an effect in women, since only 3% of women smoked during the week prior to, and <1% during the week of the procedure, whereas 6% of men smoked the week prior to, and 4% during the week of the procedure. Therefore, an effect for women may have been evident if there was a larger number of women who smoked the week before. Finally, the quantity (e.g. amount) and duration (e.g. recent, long-term) of smoking was greater on average, for men.

A retrospective study (Joesbury *et al.*, 1998) of 498 couples investigated the effects of male and female smoking at the first patient consultation (e.g. past tobacco exposure, with no quantity or duration measures) on the likelihood of achieving an ongoing pregnancy at 12 weeks; among male smokers there was a decrease of 2.4% in the chances of achieving a 12-week pregnancy with every 1-year increase in age. Another study (Hughes and Brenna, 1996) found that neither male nor female smoking affected the achievement of a clinical pregnancy; it was postulated this may have occurred because smoking is only associated with pregnancy loss following early clinical detection of a pregnancy (Joesbury *et al.*, 1998).

The current study followed men until the pregnancy outcome, and found no statistically significant effects of smoking on achieving a pregnancy, or on pregnancy outcome. However, for men who smoked during their lifetime, there was a 5.42-fold higher risk (95% CI 1.01–29.19, $P = 0.05$) of multiple births during IVF or GIFT, while adjusting for the woman's age, attempt number and number of embryos transferred. The biological plausibility for this result is difficult to explain. One study (Parazzini *et al*., 1996) previously reported OR for dizygotic and monozygotic pregnancies of 1.4 and 2.4 respectively for women smoking >10 cigarettes per day, but the trend in risk with number of cigarettes smoked per day and duration of the habit was not significant. To date, no studies have been published which link male smoking to an increased risk of multiple births.

The unique contributions of the current study were that: (i) it was prospective; (ii) it examined the effects of smoking (and passive smoking) on the success rates of all endpoints of IVF and GIFT, including live births and health of the infant; (iii) it obtained accurate and detailed smoking histories (focusing on timing and duration of exposure) by questionnaires from both female and male partners, beginning at the first clinic visit until the final pregnancy outcome; (iv) it included couples of Asian, African-American and Hispanic races; and (v) it tested for significant interactions and adjusted for multiple potential confounders, including age, lifestyle habits, reproductive history and technical aspects of the IVF and GIFT procedures.

Although the current study provides strong support that smoking can negatively impact pregnancy outcomes for couples undergoing IVF or GIFT, it did have some limitations. Ideally, we should have obtained data on serum cotinine concentrations; the validity of self-reported smoking without knowledge of cotinine and/or nicotine concentrations was a potential source of bias in our study, as cotinine (which is a metabolite of nicotine and has a serum half-life of 10–24 h) is a reliable indicator of exposure to smoking. However, in another study which compared self-reported smoking status with biochemical validation, there were high levels of sensitivity and specificity for self-report (87 and 89% respectively) (Patrick *et al.*, 1994). The infertile couples seeking treatment may not have been representative of all infertile couples with regard to their smoking habits (Augood *et al.*, 1998). Moreover, not all couples who were approached agreed to participate, and this may have resulted in a selection bias as we were unable to provide characteristics of non-respondents. The demographic characteristics of women who sought assisted reproductive treatment may have differed significantly from those women who chose not to use the services (Hirsch and Mosher, 1987). Finally, a valid criticism of statistical inference in this study—as well as any study with multiple endpoints and many main effects—is that associations found between the effects and outcomes may be spurious and attributable to chance, rather than to any 'real' association. There is little consensus among biostatisticians on how to correct for this problem. If all the null hypotheses being tested were independent, then a Bonferroni- or Tukey-type adjustment could be made (Miller, 1986; Rice, 1988). However, in the current study the various endpoints are correlated: the number of oocytes retrieved is clearly related to the number of fertilizations and to the pregnancy outcome. This leads to many dependent null hypotheses, and hence, a stringent Bonferroni adjustment would be inappropriate (Miller, 1986; Rice, 1988). We concede that this is a possible limitation in our conclusions, but make the case that our results highlight clear trends that smoking detrimentally affects pregnancy outcome. We consider it a strength of our study that the effects of smoking on all biological endpoints were evaluated to uncover the entire sequence of outcomes that constitutes the reproductive process. Future studies should be designed, based on the evidence provided herein, to replicate our findings.

In the current study, there were 221 women, 47% of whom were lifetime smokers, and the success rates for IVF and GIFT among the non-smokers and smokers were 44 and 24% respectively, resulting in 86% power to detect a relative risk of 2.5; the high success rates reflect the expertise of the sites. Although there was sufficient power to detect differences in pregnancy rates among smokers and non-smokers, very few women smoked during the month, week, or day prior to, as well during the IVF or GIFT visit. Hence, there may have been a lack of power to detect a dose–response effect.

The knowledge gained from these results regarding the effects of male and female smoking on IVF and GIFT procedures may help to create guidelines for clinicians, greatly increase success rates, and provide a forceful impetus for both men and women undergoing IVF or GIFT to stop smoking. Furthermore, our findings may ultimately have broader implications for all couples of reproductive age, and may possibly shed light on the effects of smoking throughout the natural continuum of reproduction, from conception to delivery.

Acknowledgements

We would like to thank Drs Michael Kettel, Jane Frederick, Eric Surrey, Snunit Ben-Ozer and Michael Vermesh for their assistance with this research. This research was supported by grant number 4RT0032 from the University of California, Office of the President, Tobacco-Related Disease Research Program.

References

American Society for Reproductive Medicine/Society for Assisted Reproductive Technology (1999) Assisted reproductive technology in the United States: 1996 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. *Fertil. Steril*., **71**, 798–807.

Augood, C., Duckitt, K. and Templeton, A. (1998) Smoking and female infertility: review and meta-analysis. *Hum Reprod.*, **13**, 1532–1539.

Elenbogen, A., Lipitz, S., Mashiach, S. *et al*. (1991) The effect of smoking on the outcome of in-vitro fertilization–embryo transfer. *Hum. Reprod*., **6**, 242–244.

El Nemr, A., Shawaf, T., Sabatini, L. *et al*. (1998) Effect of smoking on ovarian reserve and ovarian stimulation in in-vitro fertilization and embryo transfer. *Hum. Reprod.*, **13**, 2192–2198.

Feichtinger, W., Papalambrou, K., Poehl, M. *et al*. (1997) Smoking and *in vitro* fertilization: a meta-analysis. *J. Assist. Reprod. Genet.*, **14**, 596–599.

Grainger, D.A. and Tjaden, B.L. (2000) Assisted reproductive technologies. In Goldman, M.B. and Hatch, M.C. (eds), *Women and Health*. Academic Press, San Diego, CA.

Harrison, K.L., Breen, T.M. and Hennessey, J.F. (1990) The effect of patient smoking habit on the outcome of IVF and GIFT treatment. *N. Z. J. Obstet. Gynaecol*., **30**, 340–342.

Hirsch, M. and Mosher, W. (1987) Characteristics of infertile women in the United States and their use of infertility services. *Fertil. Steril.*, **47**, 618–625.

Hughes, E. and Brenna, B. (1996) Does cigarette smoking impair natural or assisted fecundity? *Fertil. Steril.*, **66**, 679–689.

Hughes, E., Young Lai, E. and Ward, S. (1992) Cigarette smoking and outcomes of in-vitro fertilization and embryo transfer: a prospective cohort study. *Hum Reprod.*, **7**, 358–361.

Joesbury, K., Edirisinghe, W., Phillips, M. *et al*. (1998) Evidence that male smoking affects the likelihood of a pregnancy following IVF treatment: application of the modified cumulative embryo score. *Hum. Reprod.*, **13**, 1506–1513.

Jones, H.W., Acosta, A.A., Andrews, M.C. *et al*. (1983) What is a pregnancy? A question for programs of *in vitro* fertilization. *Fertil. Steril.*, **40**, 728.

Kleinbaum, D., Kupper, L. and Morganstern, H. (1982) *Observational Research.* Lifetime Learning Publishers, CA.

Mailhes, J., Young, D., Caldito, G. *et al*. (2000) Sensitivity of mouse oocytes to nicotine-induced perturbations during oocyte meiotic maturation and aneuploidy *in vivo* and *in vitro*. *Mol. Hum Reprod.*, **6**, 232–237.

Maximovich, A. and Beyler, S. (1995) Cigarette smoking at time of *in vitro* fertilization initiation has negative effect on *in vitro* fertilization-embryo transfer success. *J. Assist. Reprod. Genet*., **12**, 75–77.

Miller, R.G. (1986) *Beyond Anova: Basics of Applied Statistics.* Wiley, New York.

Padilla, S.L. and Garcia, J.E. (1989) Effect of maternal age and number of *in vitro* fertilization procedures of pregnancy outcome. *Fertil. Steril.*, **52**, 270–273.

Parazzini, F., Chatenoud, L., Benzi, G. *et al*. (1996) Coffee and alcohol intake, smoking and risk of multiple pregnancy. *Hum. Reprod.*, **11**, 2306–2309.

Patrick, D., Cheadle, A. and Thompson, D. (1994) The validity of self-reported smoking: a review and meta-analysis. *Am. J. Public Health*, **84**, 1086–1093.

Pattison, H.A., Taylor, P.J. and Pattison, M.H. (1991) The effect of cigarette smoking on ovarian function and early pregnancy outcome of *in vitro* fertilization treatment. *Fertil. Steril.*, **55**, 780–783.

Rice, J.A. (1988) *Mathematical Statistics and Data Analysis.* Wadsworth & Brooks/Cole, Monterey, CA.

Rosevear, S., Holt, D., Lee, T. *et al*. (1992) Smoking and decreased fertilization rates *in vitro*. *Lancet*, **340**, 1995–1996.

Seibel, M.M. (1980) *Infertility: A Comprehensive Text.* Appleton & Lange, East Norwalk, Connecticut, MA.

Session, D., Hammitt, D., Damario, M. *et al*. (1998) Recent advances in infertility treatment. *Minn. Med.*, **81**, 27–32.

Shahara, F., Beatse, S. and Leonardi, M. (1994) Cigarette smoking accelerates the development of diminished ovarian reserve as evidence by the clomiphene citrate challenge test. *Fertil. Steril*., **62**, 257–262.

Sterzik, K., Strehler, E., De Santo, M. *et al*. (1996) Influence of smoking on fertility in women attending an *in vitro* fertilization program. *Fertil. Steril*., **65**, 810–830.

Stillman, R.J., Rosenberg, M.J. and Sacks, B.P. (1986) Smoking and reproduction. *Fertil. Steril.*, **46**, 545–566.

Taymor, M.L. (1990) *Infertility: A Clinician's Guide to Diagnosis and Treatment.* Plenum Publishing Corporation, New York.

The American Fertility Society (1989) Medical Research International and the Society of Assisted Reproductive Technology, The American Fertility Society: *In Vitro* Fertilization/Embryo Transfer in the United States: 1987 Results From the National IVF-ET Registry. *Fertil. Steril*., **51**, 13–18.

Trapp, M., Kemeter, P. and Feichtinger, W. (1986) Smoking and in-vitro fertilization. *Hum. Reprod.*, **1**, 357–358.

Van Voorhis, B., Dawson, J., Stovall, D. *et al*. (1996) The effect of smoking on ovarian function and fertility during assisted reproduction. *Obstet. Gynecol.*, **88**, 785–791.

Weigert, M., Hofstetter, G., Kaipl, D. *et al*. (1999) The effect of smoking on oocyte quality and hormonal parameters of patients undergoing *in vitro* fertilization-embryo transfer. *J. Assist. Reprod. Genet.*, **16**, 287–293.

Weiss, T. and Eckert, A. (1989) Cotinine levels in follicular fluid and serum of IVF patients: effect on granulosa-luteal cell function *in vitro*. *Hum. Reprod.*, **4**, 482–485.

Williams, J. (1985) *Obstetrics.* Appleton, New York.

Yuzpe, A.A., Brown, S.E., Casper, R.F *et al*. (1983) Rates and outcome of pregnancies achieved in the first four years of an in-vitro fertilization program. *Can. Med. Assoc. J*., **140**, 167–172.

Zenzes, M. (2000) Smoking and reproduction: gene damage to human gametes and embryos. *Hum. Reprod. Update*, **6**, 122–131.

Zenzes, M. and Reed, T. (1997) Effects of cigarette smoking and aging on maturation of human oocytes. *Hum. Reprod.*, **12**, 1736–1741.

Zenzes, M., Wang, P. and Casper, R. (1995) Cigarette smoking may affect meiotic maturation of human oocytes. *Hum. Reprod.*, **10**, 3213–3217.

Zenzes, M., Puy, L. and Bielecki, R. (1998) Immunodetection of benzo[a]pyrene adducts in ovarian cells of women exposed to cigarette smoke. *Mol. Hum. Reprod.*, **4**, 159–165.

Received on October 27, 2000; accepted on March 20, 2001