Evidence that male smoking affects the likelihood of a pregnancy following IVF treatment: application of the modified cumulative embryo score

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Female cigarette smoking has been implicated as having a detrimental effect on in-vitro fertilization (IVF) outcomes mediated through: (i) a diminished ovarian reserve (DOR), and (ii) an elevated pregnancy loss. Research is sparse regarding the effect of male smoking. The objective of this retrospective cohort study was to investigate the effect of male and female smoking on: (i) the collective quality of embryos selected for uterine transfer, and (ii) the likelihood of achieving an ongoing pregnancy at 12 weeks. A total of 498 consecutive IVF treatment cycles were analysed. Female smokers were significantly younger ($P < 0.05$) and achieved a better modified cumulative embryo score (mCES) ($P < 0.05$) than female non-smokers. Female age correlated inversely with the number of oocytes collected ($r = -0.42$, $P < 0.01$) and the number of oocytes in turn was important in terms of predicting mCES. The decreasing number of oocytes aspirated with increasing age was of a significantly stronger magnitude for female smokers than for female non-smokers ($P < 0.05$). Multiple logistic regression was used to determine whether smoking affected the likelihood of achieving a 12-week pregnancy. The mCES, tubal infertility and male smoking were found to be significant. Male smoking interacted with male age ($P = 0.0164$), indicating for male smokers a decrease of 2.4% in the likelihood of achieving a 12-week pregnancy with every 1-year increase in age. This is the first study to show that male smoking has a deleterious effect on pregnancy outcome among IVF patients. Our study supports the increased risk of DOR but fails to support the elevated incidence of pregnancy loss among female smokers. A reduced pregnancy rate was associated with male smoking possibly through pre-zygotic genetic damage. The growing realization of a paternal component of reproductive impairment suggests that studying the male is necessary.
Key words: embryo quality/IVF/mutagenicity/pregnancy loss/smoking

Introduction

The impact of smoking on the clinical outcomes of in-vitro fertilization (IVF) treatment has yet to be resolved. Collectively the studies indicate that female smoking has a small but significant detrimental effect (Hughes and Brennan, 1996). The evidence implicates two mechanisms whereby female smoking may exert this effect: a diminished ovarian reserve resulting in the production of fewer oocytes (Sharara *et al.*, 1994; Van Voorhis *et al.*, 1996), and an elevated incidence of pregnancy loss (Harrison *et al.*, 1990; Pattinson *et al.*, 1991; Maximovich and Beyler, 1995). Research is sparse regarding the effect of male smoking but what is available suggests that male smoking does not significantly affect IVF outcomes (Pattinson *et al.*, 1991; Hughes *et al.*, 1994).

The objective of this study was to investigate the effect of male and female smoking on: (i) the collective quality of embryos selected for uterine transfer, and (ii) the likelihood of achieving an ongoing pregnancy at 12 weeks.

Materials and methods

This retrospective cohort study includes 498 consecutive IVF treatment cycles from 385 couples performed between January 1994 and December 1995. The data were obtained from patient clinical outcome records and patient files. In-vitro fertilization–embryo transfer (IVF–embryo transfer) and intracytoplasmic sperm injection–embryo transfer (ICSI–embryo transfer) cycles were included in the study. Treatment cycles involving donor sperm, donor oocytes or donor embryos were excluded.

Smoking status

Female and male smoking status was obtained from the patients' files, which was typically recorded at the patient's first consultation at the clinic. Smoking status was coded for analysis as 'smoker' or 'non-smoker'. Non-smokers included 'never' and 'ex-smokers'. The average number of cigarettes smoked per day was missing for a high proportion of the patients recognized as smokers and this precluded the use of such a smoking exposure variable in the data analysis.

Female patients received subcutaneous injections of gonadotrophin-releasing hormone (GnRH) analogue, leuprolide acetate (Lucrin; Abbott, Kurnell, NSW, Australia), 1 mg (20 IU) daily, either from day 21 of the previous menstrual cycle (pituitary down-regulation) or from day 1 of the treatment cycle ('flare' effect). Human menopausal gonadotrophin (Pergonal; Serono, Frenchs Forest, NSW) was given to all the female patients from day 3 of the treatment cycle, with an increase in the dosage depending on the oestradiol rise. Ovulation was triggered with 10 000 IU of human chorionic gonadotrophin (HCG) and oocytes were aspirated transvaginally by ultrasound guidance 35 h after the HCG trigger.

Oocytes and embryos were cultured in human tubal fluid medium supplemented with 10% heat-inactivated patient's serum. Insemination was carried out 4–6 h after oocyte recovery with 50 000–100 000 spermatozoa/ml. The presence of two pronuclei 18–20 h after insemination confirmed that normal fertilization had occurred.

The texture and thickness of the endometrium was assessed on the

day of or within 1 or 2 days of the HCG trigger by transvaginal ultrasound. The maximum thickness of the endometrium on both sides of the midline was measured in the plane through the central longitudinal axis of the uterine body (Gonen and Casper, 1990). The endometrium was classified as: A, an entirely homogeneous, hyperechogenic endometrium; B, an intermediate type characterized by the same reflectivity of ultrasound as the myometrium, with a non-prominent or absent central echogenic line; or C, a multilayered endometrium consisting of prominent outer and midline hyperechogenic lines and inner hypoechogenic regions (Gonen and Casper, 1990).

Embryo quality
Embryos were graded on the second day following insemination just prior to transfer. Grading was based on granularity and symmetry of the blastomeres, fragmentation and rate of development. A hypothetically perfect embryo was graded a maximum of 4.0 points with 0.5 or 1.0 point deducted in accordance with the degree of deviation from the optimum for each morphological parameter (Yovich and Lower, 1991). The highest scoring embryos were selected for uterine transfer.

The collective quality of embryos selected for transfer was based on the cumulative embryo score (CES) devised by Steer *et al.* (1992), which entailed multiplying the grade of each embryo selected for transfer by the number of cells and then summing these values. However, embryos developing faster than the 'ideal' growth rate (Trounson *et al.*, 1982; Cummins *et al.*, 1986) have been shown to have pregnancy outcomes that are poorer than embryos that exhibit a 'normal' growth pattern (Cummins *et al.*, 1986; Giorgetti *et al.*, 1995). To account for the potential inferiority of fast-developing embryos, we modified the manner in which the CES was calculated. For embryos at the 5-cell stage, we awarded 3 points for number of cells and for 6-, 7-, or 8-cell embryos, we awarded 2 points for number of cells. This modified cumulative embryo score (mCES) was used as a measure of the collective quality of the embryos selected for uterine transfer.

Pregnancy outcome
The principal pregnancy outcome variable was an ongoing pregnancy at 12 weeks, which included pregnancies that had reached 12 weeks of gestation from the commencement of the last menstrual period and excluded any prior pregnancy losses including biochemical, blighted ovum, ectopic pregnancy and spontaneous miscarriage. Other pregnancy outcomes examined were a positive β-HCG pregnancy test, clinical pregnancy and a live birth. A positive β-HCG test was characterized by an elevated serum β-HCG level on or after day 16 of the luteal phase, with a significant rise at least 3 days thereafter (Yovich *et al.*, 1986). The assay for β-HCG had a minimum detection level of 2.5 IU/l and all levels of 25 IU/l were standardized against the 2nd International Standard (61/6). Serum oestradiol and progesterone were also required to be in the appropriate pregnancy range of >550 pmol/l and >37 nmol/l, respectively. A clinical pregnancy was defined as ultrasonographic evidence of fetal heart activity or chorionic villi evidence after a spontaneous abortion or an ectopic pregnancy. A live birth included all pregnancies with at least one live birth of which an infant was alive one month post-delivery.

Statistical analysis
Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows (Release 7.0). The treatment cycle was treated statistically as an independent event. We acknowledge that this practice is open to question and needs to be addressed in future studies involving IVF patients. Independent sample *t*-tests were used to compare the means of two groups. The Pearson χ^2 was used to test the difference between two proportions and between two categorical variables. The Student's *t*-test was used to compare the difference between the simple linear regression coefficients (Zar, 1974). To test the effect of multiple factors on a continuous outcome variable, multiple linear regression was applied. Logistic regression was used to investigate the effect of multiple factors on pregnancy outcomes. Backward elimination was conducted to first construct a model which comprised only the significant independent variables. For each of these variables, interaction terms were constructed from all the original independent variables. Each interaction term was examined in the final model in a forward fashion.

Results
Of the 498 treatment cycles, smoking status was ascertained for 465 of the females (93.4%) and for 422 of the males (84.6%). Based on this, 76 of the female patients (15.9%) and 101 of the male patients (20.3%) were smokers. Smoking status was determined for 415 of the couples (83.3%): for 287 couples both partners were non-smokers (69.2%), for 30 couples only the female smoked (7.2%), for 59 couples only the male smoked (11.8%), and for 39 couples both partners were smokers (9.4%). With regard to the number of embryos transferred, 82.7% of cycles involved the transfer of three embryos, 11.6% two embryos, 3.2% one embryo, and in 2.4% of treatment cycles, four embryos were transferred.

Smokers versus non-smokers
Female smokers were compared with female non-smokers in terms of age, serum oestradiol levels, number of oocytes retrieved, proportion of oocytes fertilized and mCES (Table I). Female smokers were shown to be significantly ($P < 0.05$) younger than female non-smokers by an average of 18 months. No significant differences were demonstrated among the other female factors examined. For males, age, fertilization rates and mCES were not significantly different among smokers and non-smokers.

Differences in infertility aetiology were examined by smoking status and sex (Table II). The difference in the proportion of tubal infertility cases among the female smokers and non-smokers was not of significance. The proportion of ICSI cycles undertaken by male smokers was not significantly different from that of male non-smokers. No difference was noted with regard to the grade or thickness of the endometrium between female smokers and female non-smokers (Table II). Female smoking was significantly associated with male smoking ($\chi^2 = 49.68$, df 1, $P < 0.001$) with over half of the female smokers (56.5%) having a male partner that smoked.

Smoking and the mCES
The number of oocytes retrieved, the percentage of oocytes that fertilized, the number of embryos transferred, female smoking and the treatment cycle number were shown to be significant predictors of the mCES (Table III). The adjusted R^2 was 45.0% which shows that almost half of the variability in the mCES was explained by the collective variability of the significant independent variables in the final model.

The β-coefficient for the number of oocytes retrieved was 0.34 [95% confidence interval (CI): 0.24–0.43] indicating that,

Table I. Continuous variables, mean $\pm$ SD by sex and smoking status

	Variable	Smokers	Non-smokers	Total
Female	Age (years)	33.1 $\pm$ 4.7[a]	34.6 $\pm$ 4.7	34.1 $\pm$ 4.7
	Serum oestradiol level (pmol/l)[b]	9250 $\pm$ 4143	9019 $\pm$ 4162	9039 $\pm$ 4124
	No. of oocytes retrieved	13.1 $\pm$ 7.7	12.2 $\pm$ 6.8	12.3 $\pm$ 6.7
	Individual proportion of oocytes fertilized	0.60 $\pm$ 0.2	0.61 $\pm$ 0.2	0.60 $\pm$ 0.2
	mCES	29.7 $\pm$ 9.1	27.4 $\pm$ 9.0	27.6 $\pm$ 9.3
	Number	74	391	465
Male	Age (in years)	36.2 $\pm$ 5.7	36.5 $\pm$ 6.3	36.6 $\pm$ 6.1
	Individual proportion of oocytes fertilized	0.61 $\pm$ 0.2	0.61 $\pm$ 0.2	0.60 $\pm$ 0.2
	mCES	28.5 $\pm$ 8.1	26.9 $\pm$ 9.3	27.6 $\pm$ 9.3
	Number	101	321	422

[a]$P < 0.05$ level.
[b]On the day of or within 1 day of the human chorionic gonadotrophin administration/trigger.
mCES = modified cumulative embryo score.

Table II. Categorical variables and distribution by sex and smoking status

	Variable	Levels (%)	Smokers (%)	Non-smokers (%)	Total
Female	Female tubal infertility aetiology	Tubal only	28.4	25.6	26.0
	Grade of endometrium	A	21.7	19.4	19.8
		B	71.0	68.7	69.0
		C	7.2	12.0	11.2
	Thickness of endometrium	<10 mm	20.0	23.6	23.0
		10–12 mm	55.7	53.8	54.2
		13+ mm	24.3	22.5	22.8
Male	Type of treatment cycle	ICSI	22.8	24.9	24.4

ICSI = intracytoplasmic sperm injection.

Table III. Final multiple linear regression model evaluating the factors affecting the mCES[a]

Variable parameters in final model		Regression coefficient	95% CI (LCL, UCL)	Significance (P-value)
No. of oocytes retrieved		0.34	0.24, 0.43	<0.001
Proportion of oocytes that fertilized		10.55	7.56, 13.53	<0.001
No. of embryos transferred:	1 vs 3	–20.2	–23.7, –16.7	<0.001
	2 vs 3	–8.5	–10.6, –6.5	<0.001
	4 vs 3	6.0	2.0, 10.0	0.003
Female smoking (no/yes)		2.38	0.68, 4.09	0.006
Treatment cycle no.		–0.47	–0.04, –0.91	0.034

[a]Covariates available to the model included female age (years), male age (years), in-vitro fertilization (IVF)–embryo transfer versus IVF-embryo transfer-intracytoplasmic sperm injection treatment cycles, female diagnosis of tubal infertility only versus all other conditions including tubal plus an additional condition, number of oocytes retrieved, proportion of oocytes that fertilized, treatment cycle number, day of human chorionic gonadotrophin trigger, female smoking status (non-smokers versus smokers), male smoking status (non-smokers versus smokers) and number of embryos transferred.
mCES = modified cumulative embryo score; CI = confidence interval; LCL = lower confidence limit; UCL = upper confidence limit.

for every three additional oocytes collected, there was an incremental increase in the mCES of approximately 1 unit when all other variables were held constant. The β-coefficient for the proportion of oocytes fertilized was 10.55 (95% CI: 7.56–13.53) demonstrating that, for every 10% of additional oocytes fertilized, there was on average an increase in the mCES of 1 point. There was an inverse relationship between the mCES and number of treatment cycles in that, on average,

there was a decrease of ~0.5 points in the mCES for every additional treatment cycle attempted. The number of embryos transferred significantly affected the mCES score. In relation to a transfer of three embryos, the mCES of a two-embryo transfer was on average 8.5 points lower, and a one-embryo transfer was on average 20.2 points lower. By contrast, a transfer of four embryos was on average 6.0 points higher than a three-embryo transfer. This interpretation assumes that
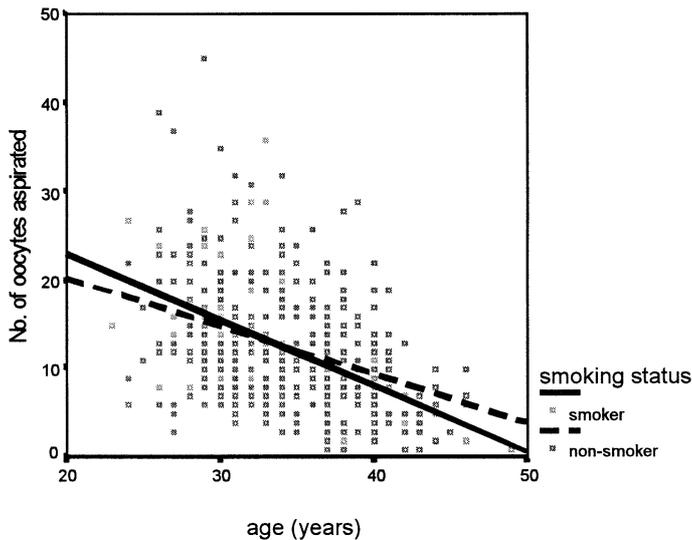
Figure 1. Relationship between female age and number of oocytes aspirated by smoking status.

the number of embryos transferred is independent of embryo quality. In practice, this may not be the case as the number of embryos selected for transfer may be dependent upon the quality of the embryos.

Female smokers on average had a mCES which was 2.4 (95% CI: 0.7–4.1) points higher than that of female non-smokers. Male smoking status was shown to be of no significance in relation to the mCES. Also of note was the non-significant effect of female age on the mCES. On further investigation it was demonstrated that female age was inversely correlated with number of oocytes collected ($r = -0.42$, $P < 0.01$). This relationship was of a stronger magnitude for female smokers than for female non-smokers (Figure 1). Simple linear regression analysis for female smokers and non-smokers was performed to assess the relationship between age and number of oocytes retrieved. The regression coefficient represents the linear relationship between female age and number of oocytes retrieved (the slope of the line). The regression coefficient for the female smokers was shown to differ significantly from that of the non-smokers ($P < 0.05$). For female smokers, there was a significant ($P < 0.001$) reduction of 0.75 (95% CI: –0.40 to –1.10) oocytes per 1-year increase in age compared with 0.55 (95% CI: –0.42 to –0.68) oocytes per 1-year increase in age for female non-smokers ($P < 0.001$).

The crude pregnancy rates for positive β-HCG pregnancy test, ongoing pregnancy at 12 weeks and live birth by smoking status are shown in Figure 2. For males and females, no significant difference ($P > 0.05$) between the pregnancy rates of smokers and non-smokers was evident.

Smoking and pregnancy

The mCES, tubal infertility and male smoking were shown to be significant factors in relation to the likelihood of achieving an ongoing pregnancy of 12 weeks (Table IV). Moreover, male smoking was shown to interact with male age. The final logistic model was as follows:

$$P = 1/1 + \exp^{-(u)}$$

where $u = -3.7719 + (0.0742 \times mCES) + (0.6294 \times tubal) - (0.0247 \times mas)$; P = probability of a pregnancy at $\geqslant 12$ weeks; mCES = modified cumulative embryo score; tubal = female tubal infertility aetiology (1 when the reason for female infertility was only tubal, and 0 when the reason for female infertility was 'other' including tubal infertility plus an additional reason); and mas = male age×male smoking (1 when the male was a 'smoker' and 0 when the male was a 'non-smoker').

The odds ratio for mCES was 1.077 (95% CI: 1.040–1.115), which indicates that, for an increase of 1 point in the mCES, the probability of pregnancy increases by 8% when all other factors are held constant. For women with tubal infertility, the odds ratio of achieving a 12-week pregnancy was 1.876 (95% CI: 1.052–3.346). Therefore, women with tubal infertility are nearly two times more likely to reach a 12-week pregnancy than women with any other classification of infertility.

The odds ratio for the interaction between male smoking and male age was 0.976 (95% CI: 0.956–0.9955). This means that, for couples where the male partner is a smoker, for every 1-year increase in the age of the male, there is a decrease of 2.4% in the likelihood of achieving a 12-week pregnancy. In other words, a 31-year-old male smoker's partner has a likelihood of achieving a 12-week pregnancy which is 97.6% of that of a 30-year-old male, or a 40-year-old male smoker's partner is 24% less likely to obtain a 12-week pregnancy than a 30-year-old male smoker's partner. For male non-smokers, male age does not affect the likelihood of their partner achieving a 12-week pregnancy.

Logistic regression analysis was repeated using clinical pregnancy as the dependent variable. The mCES and tubal infertility were found to be significant ($P < 0.001$ and $P = 0.0057$, respectively), however, male and female smoking were non-significant. Female smoking was removed from the model with a P-value of 0.99 and male smoking with a P-value of 0.54.

Discussion

Smoking and the mCES

We modified the CES to account for the potential inferiority of fast-developing embryos. The mCES was shown to be significantly associated with female but not male smoking. Female smokers experienced the transfer of significantly better-quality embryos than female non-smokers by an average of 2.4 points. However, female smokers were significantly younger ($P < 0.05$) than their non-smoking counterparts. In clinical practice, a proportion of treatment cycles are cancelled prior to embryo transfer. Approximately one-third of the 3093 treatment cycles commenced in the study by Haan *et al.* (1991) failed to result in an embryo transfer. It is possible that female smokers are over-represented among these cancelled cycles, especially older women who have long-term smoking histories. This age discrepancy supports our hypothesis that older female smokers are less likely to achieve an embryo transfer and are under-represented in our study population.

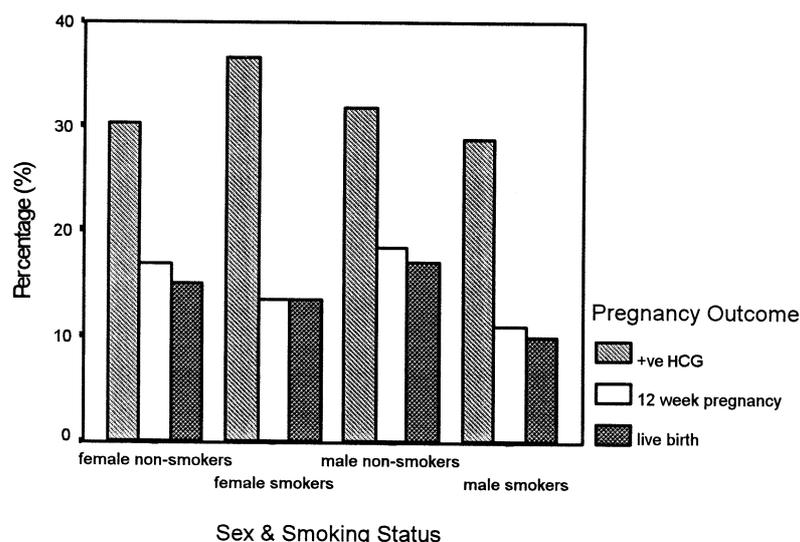Cigarette smoking appears to interfere with ovarian follicular

Figure 2. Crude pregnancy rates by sex and smoking status.

Table IV. Final logistic regression model evaluating the factors affecting 12-week ongoing pregnancy

Variable parameters in final model	Odds ratio	95% CI (LCL, UCL)	Significance (*P*-value)
mCES	1.077	1.040, 1.115	<0.0001
Female tubal infertility only (no/yes)	1.876	1.052, 3.346	0.0329
Male smoking (no/yes) by male age (years)	0.976	0.956, 0.996	0.0164

Covariates available to the model included mCES, female age (years), male age (years), in-vitro fertilization (IVF)–embryo transfer versus IVF–embryo transfer-intracytoplasmic sperm injection treatment cycles, female diagnosis of tubal infertility only versus all other conditions including tubal plus an additional condition, oestradiol levels on day of human chorionic gonadotrophin (HCG) trigger or within 1 day of HCG trigger, vascular grade of endometrium (A, B, C), endometrium thickness (<10 mm, 10–12 mm, ≥13 mm), female smoking status (non-smokers versus smokers), and male smoking status (non-smokers versus smokers). mCES = modified cumulative embryo score; CI = confidence interval; LCL = lower confidence limit, UCL = upper confidence limit.

maturation and oocyte production, as female smokers tend to produce fewer oocytes than female non-smokers (Harrison *et al.*, 1990; Van Voorhis *et al.*, 1992). Van Voorhis *et al.* (1996) showed that for every 10-pack-years of cigarette smoking, 2.5 fewer mature oocytes and two less embryos were obtained from female smokers. Pack-years were defined as the number of packs of cigarettes smoked per day times the number of years a women smoked. Sharara *et al.* (1994) reported that the incidence of diminished ovarian reserve (DOR) is higher among female smokers than non-smokers. DOR is characterized by elevated levels of follicle stimulating hormone resulting in a decreased ovarian response (Navot *et al.*, 1987). Consistent with these findings, we demonstrated that the decline in the number of oocytes with age occurs at a significantly faster rate among smokers than non-smokers. For female smokers, there was a reduction in oocytes of 0.75 (95% CI: 0.4–1.1) for every 1-year increase in age compared with a reduction of 0.55 (95% CI: 0.4–0.7) per year for non-smokers. Our study, like these other studies, supports the association between female smoking and early menopause (Jick *et al.*, 1977).

Smoking and pregnancy

When investigating the effect of smoking on pregnancy outcome, it is important to consider possible biological mechan-

isms that may account for an effect and to choose a pregnancy outcome accordingly. It has been postulated that cigarette smoke may interfere with the DNA of the gametes and in turn may affect the developing embryos (Wyrobek, 1993). We selected ongoing pregnancies at 12 weeks as the pregnancy outcome, as over 90% of the pregnancy losses in our sample occurred within the first 12 weeks after the last menstrual period. Therefore, a pregnancy that reaches the 12-week stage has a good chance of proceeding to a live birth.

Female smoking was not shown to reduce the likelihood of achieving a 12-week pregnancy. However, others have shown a reduction in crude pregnancy rates among female non-smokers compared with those of female non-smokers (Harrison *et al.*, 1990; Pattinson *et al.*, 1991). The findings of these studies are difficult to interpret as crude pregnancy rates do not take into account important group differences such as age, embryo quality and infertility status. A few other studies have used multivariate analysis techniques to assess the effects of smoking. A cohort study involving 499 female IVF patients reported a reduction of 50% in the ongoing pregnancy rate of female smokers in relation to female non-smokers (Van Voorhis *et al.*, 1996). By contrast, Hughes *et al.* (1994) found that neither male nor female smoking affected the likelihood of a clinical pregnancy. The issue as to whether female smoking

impacts on pregnancy outcome, in addition to the alleged reduction of oocytes produced, needs further clarification.

Male smoking was shown to have a significant negative effect on pregnancy outcome, and the effect was dependent on the age of the male smoker. For male smokers, every 1-year increase in age was associated with a reduction of 2.4% in the probability of their partner achieving a 12-week pregnancy. It is plausible that male age is a surrogate measure of duration of smoking exposure in that it is an indirect measure of the number of years the male has been a smoker. This finding suggests that there is a dose response relationship between male smoking and pregnancy outcome, thus supporting the potential causality of the relationship.

To the authors' knowledge, this is the first study to implicate male smoking as having a detrimental effect on pregnancy outcome following IVF treatment. The smoking status of the male partner was not controlled for in the study by Van Voorhis *et al.* (1996), who reported a 50% reduction in the pregnancy rate of female smokers. We found, as did Hughes *et al.* (1994), that male smoking was associated with female smoking and this potentially confounding effect cannot be eliminated as an explanation for the observed difference in pregnancy rates between female smokers and non-smokers in the Van Voorhis *et al.* (1996) study. Hughes *et al.* (1994) reported that male smoking did not affect the rate of clinical pregnancies. A limitation of their study was the choice of clinical pregnancies as the pregnancy outcome. Pregnancy losses following clinical pregnancy detection may be linked to male smoking. We found that male smoking was not significant in relation to clinical pregnancies. This raises the suggestion that male smoking may be associated with pregnancy loss following early clinical detection of a pregnancy.

Biological plausibility

Over 60 chemical compounds have been identified in tobacco smoke which are carcinogenic and/or mutagenic (Bos and Henderson, 1984). Cotinine, a tobacco specific metabolite, has been detected in the follicular fluid of female smokers (Weiss and Eckert, 1989; Zenzes *et al.*, 1996). More recently, cotinine was shown to accumulate in the maturing follicles and to interact with and incorporate into the follicular cells (Zenzes *et al.*, 1997). Extracts of cigarette smoke have been shown to inhibit the conversion of androstenedione to oestradiol in cultures of human granulosa cells (Barbieri *et al.*, 1986). This in part may explain the higher incidence of immature oocytes observed among female smokers (Zenzes *et al.*, 1995). Diploid oocytes are immature and possibly result from prevention of the first polar body extrusion (Edirisinghe *et al.*, 1992; Zenzes *et al.*, 1995) or as a result of oocyte arrest at syngamy after sperm entry (Edirisinghe *et al.*, 1992). Whilst Zenzes *et al.* (1995) found that female smokers were more likely to produce immature diploid oocytes, they were unable to show any difference in the occurrence of other cytogenetic abnormalities, specifically aneuploid (19–22 and 24–27) and haploid (23) chromosome complements among female smokers. A high proportion of abortuses exhibit chromosomal abnormalities (Plachot, 1989; Yovich and Lower, 1991) of which aneuploidy

has been identified as being the most common (Hassold *et al.*, 1980).

Chemical components of tobacco smoke may induce alkylation of DNA in the oocyte (Bos *et al.*, 1989). Bos *et al.* (1989) used the *Salmonella* microsome assay to compare follicular fluid mutagenicity of female smokers and female non-smokers. They were unable to show that the mutagenicity differed significantly even though control samples of urine did show marked elevation of mutagenicity among the female smokers. They concluded that a measurable amount of tobacco smoke mutagens is absent in follicular fluid, possibly due to the difficulty mutagens have of reaching and/or penetrating the protective surrounding layers of the follicle, theca interna, basement membrane and granulosa cells.

The opportunities for mutation to occur in the maternal germ cells differ from those of the paternal germ cells. In males, spermatogenesis occurs from adolescence to an advanced age resulting in a prolonged 'window' of exposure (Little and Vainio, 1994). By contrast, the opportunities for mutation to occur in maternal germ cells may be restricted by the fact that the germ cells are almost fully mature in the ovaries of newborn females (Little and Vainio, 1994) and remain so until fertilization. Therefore, mutant genes are less likely to accumulate in the female than in the male (Cavalli-Sforza and Bodmer, 1971). The interaction between male smoking and age observed in our study lends support to this theory that mutations may arise in the paternal gametes during the preconceptional period and accumulate with time (Little and Vainio, 1994).

Our view is consistent with Wyrobek (1993) in that male reproductive health is related to reproductive potential and the health of the resultant offspring. Wyrobek (1993) proposed that there are a number of avenues whereby exposure to toxic agents may impair male reproduction. Paternally transmitted genetic defects, mutations or non-mutational alterations could result in fetal loss or abnormal development *in utero*. Reproductive potential may also be related to the spermatogonial cells' and spermatids' ability to repair DNA lesions (Zenzes, 1995) and to the capacity of the fertilized egg to repair DNA lesions in the spermatozoon (Matsuda and Tobari, 1989; Genesca *et al.*, 1992). The frequency of genetic defects, non-mutational alterations and/or sperm lesions may be related to paternal age, as may the capacity to repair sperm lesions. This in part may account for the reduction in the likelihood of a successful outcome with increasing age of male smokers.

A recent study reported that at least one-quarter of semen samples contain between 5% and 40% of spermatozoa with fragmented DNA, and that male smokers had a significantly higher percentage of DNA-fragmented spermatozoa than non-smokers (Sun *et al.*, 1997). DNA fragmentation was negatively correlated with sperm motility, morphology and count, and was associated with a reduced rate of fertilization and embryo cleavage. By contrast, spermatozoa with DNA anomalies have also been shown to have a similar fertilization potential to that of spermatozoa with normal DNA (Peluso *et al.*, 1992). In our study, the rate of fertilization did not differ between male smokers and male non-smokers, which is consistent with the findings of Peluso *et al.* (1992). This implies that the process

of fertilization is not impaired in spermatozoa with potential tobacco smoke-induced DNA damage. However, if fertilization is initiated by a spermatozoon with defective DNA, it is likely that the resulting zygote would have limited capacity for normal embryonic development. The zygote genome begins to control embryonic development around the 8- to 16-cell stage, and therefore any defect in the DNA will not be evident if the transfer occurs on the second or third day after insemination (Peluso *et al.*, 1992).

Study limitations

We acknowledge that our data on smoking status obtained at the first patient consultation may not accurately reflect cigarette consumption at the time of IVF treatment (Hughes *et al.*, 1992). The average number of daily cigarettes consumed for smokers was not readily available from the patients' files. Misclassification of smoking exposure, as may be the case in our study, is likely to generate results that favour the null hypothesis of no effect. Despite this, a negative effect of male smoking on pregnancy outcome was shown. This implies that the observed risk is an underestimate of the true effect. We are confident that our male smoking variable does represent at least past tobacco exposure even if the male had ceased smoking upon commencing treatment. Given the current public health awareness of the effects of smoking on female reproduction, smoking cessation or reduction is more likely among female patients. It is less likely that male patients will modify their smoking behaviour as little information exists to show that male smoking has a detrimental effect on reproduction, especially in relation to pregnancy loss. Despite failing to demonstrate an effect of female smoking on pregnancy, we cannot eliminate the possibility that misclassification of female smoking status obscured a true negative effect.

Conclusion

Our study supports the increased risk of DOR but fails to support a reduction in the likelihood of a 12-week pregnancy among female smokers. Rather, we found that pregnancy outcome was negatively associated with male smoking. The findings of this study add to a growing body of knowledge that shows that male smoking may cause pre-zygotic DNA damage that in turn is linked to the failure of an embryo/fetus to develop *in utero*. Reproductive problems such as fetal loss have been associated traditionally with the female partner. Consequently, research on the effect of smoking has been focused on women. The increasing awareness of a paternal component of reproductive impairment suggests that studying the male is necessary (Stillman *et al.*, 1986). We are currently undertaking a prospective cohort study investigating the effect of lifestyle factors on the clinical outcomes of IVF treatment. Specific attention is being given to the quantification of tobacco (past and present), caffeine and alcohol consumption of both the male and female partner. We anticipate that this will contribute towards clarifying whether female and male smoking affects the outcomes of IVF, and reproduction in general.

References

Barbieri, R.L., McShane, P.M., Ryan, K.J. (1986) Constituents of cigarette smoke inhibit human granulosa cell aromatase. *Fertil. Steril.*, **46**, 232–236.

Bos, R.P. and Henderson, P.Th. (1984) Genotoxic risk of passive smoking. *Reviews on Env. Hlth,* **IV**, 161–178.

Bos, R.P., van Heijst, C.H.M., Hollanders, H.M.G. *et al.* (1989) Is there influence of smoking on the mutagenicity of follicular fluid? *Fertil. Steril.*, **52**, 774–777.

Cavalli-Sforza, L.L. and Bodmer, W.F. (1971) *The Genetics of Human Populations*. Freeman, San Francisco, CA, pp. 98.

Cummins, J.M., Breen, T.M., Harrison, K.L. *et al.* (1986) A formula for scoring human embryo growth rates in *in vitro* fertilization: Its value in predicting pregnancy and in comparison with visual estimates of embryo quality. *J. In Vitro Fertil. Embryo Transfer*, **3**, 284–295.

Edirisinghe, W.R., Murch, A.R. and Yovich, J.L. (1992) Cytogenetic analysis of human oocytes and embryos in an in-vitro fertilization programme. *Hum. Reprod.*, **7**, 230–236.

Genesca, A., Caballin, M.R., Miro, R. *et al.* (1992) Repair of human sperm chromosome aberrations in the hamster egg. *Hum. Genet.*, **89**, 181–186.

Giorgetti, C., Terriou, P., Auquier, P. *et al.* (1995) Embryo score to predict implantation after in-vitro fertilization; based on 957 single embryo transfers. *Hum. Reprod.,* **10**, 2427–2431.

Gonen, Y. and Casper, R.F. (1990) Prediction of implantation by the sonographic appearance of the endometrium during controlled ovarian stimulation for *in vitro* fertilization (IVF). *J. In Vitro Fertil. Embryo Transfer,* **7**, 146–152.

Haan, G., Bernardus, R.E., Hollanders, J.M.G. *et al.* (1991) Results of IVF from a prospective multicentre study. *Hum. Reprod.*, **6**, 805–810.

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. *Aust. NZ J. Obstet. Gynaecol.*, **30**, 340–342.

Hassold, T., Chen, N., Funkhouse, J., Joose, T. *et al.* (1980) A cytogenetic study of 1000 spontaneous abortions. *Ann. Hum. Genet.*, **44**, 151–178.

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

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

Hughes, E.G., Yeo, J., Claman, P. *et al.* (1994) Cigarette smoking and the outcomes of *in vitro* fertilization: Measurement of effect size and levels of action. *Fertil. Steril.*, **62**, 807–814.

Jick, H., Parker, R. and Morrison, A.S. (1977) Relationship between smoking and age of natural menopause. *Lancet*, **1**, 1354–1355.

Little, J. and Vainio, H. (1994) Mutagenic lifestyles: A review of evidence of associations between germ-cell mutations in humans and smoking, alcohol consumption and use of 'recreational' drugs. *Mutat. Res.*, **313**, 131–151.

Matsuda, Y. and Tobari, I. (1989) Repair capacity of fertilized mouse eggs for X-ray damage induced in sperm and mature oocytes. *Mutat. Res.*, **210**, 35–47.

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

Navot, D., Rosenwaks, Z. and Margalioth, E.J. (1987) Prognostic assessment of female fecundity. *Lancet*, **8560**, 645–648.

Pattinson, H.A., Taylor, P.J. and Pattinson, 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.

Peluso, J.J., Luciano, A.A., Nulsen, J.C. (1992) The relationship between alterations in spermatozoal deoxyribonucleic acid, heparin binding sites, and semen quality. *Fertil. Steril.*, **57**, 665–670.

Plachot, M. (1989) Chromosome analysis of spontaneous abortions after IVF. A European study. *Hum. Reprod.*, **4**, 425–429.

Sharara, F.I., Beatse, S.N., Leonardi, M.R. *et al.* (1994) Cigarette smoking accelerates the development of diminished ovarian reserve as evidenced by the clomiphene citrate challenge test. *Fertil. Steril.*, **62**, 257–262.

Steer, C.V., Mills, C.L., Tan, S.L. *et al.* (1992) The cumulative embryo score: a predictive embryo scoring technique to select the optimal number of embryos to transfer in an in-vitro fertilization and embryo transfer programme. *Hum. Reprod.*, **7**, 117–119.

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

Sun, J.G., Jurisicova, A. and Casper, R.F. (1997) Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization *in vitro*. *Biol. Reprod.*, **56**, 602–607.

Trounson, A.O., Mohr, L.R., Wood, C. and Leeton, J.F. (1982) Effect of delayed insemination on *in vitro* fertilization, culture and transfer of human embryos. *J. Reprod. Fertil.*, **64**, 285–294.

Van Voorhis, B.J., Syrop, C.H., Hammitt, D.G. *et al.* (1992) Effects of smoking on ovulation induction for assisted reproductive techniques. *Fertil. Steril.*, **58**, 981–985.

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

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**, 482–482.

Wyrobek, A.J. (1993) Methods and concepts in detecting abnormal reproductive outcomes of paternal origin. *Reprod. Toxicol.*, **7**, 3–16.

Yovich, J.L., Lower, A. (1991) Implantation failure: clinical aspects. In Seppälä, M. (ed.), *Balliere's Clinical Obstetrics and Gynaecology*. Balliere Tindall, London, pp. 211–252.

Yovich, J.L., Wilcox, D.L., Grudzinskas, J.G. and Bolton, A.E. (1986) The prognostic value of hCG, PAPP-A, oestradiol-17β and progesterone in early human pregnancy. *Aust. NZ J. Obstet. Gynaecol.*, **26**, 59–64.

Zar, J.H. (1974) *Biostatistical Analysis*. Prentice-Hall: Englewood Cliffs, New Jersey.

Zenzes, M.T. (1995) Cigarette smoking as a cause of delay in conception. *Reprod. Med. Rev.*, **4**, 189–205.

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

Zenzes, M.T., Reed, T.E., Wang, P. and Klein, J. (1996) Cotinine, a major metabolite of nicotine, is detectable in follicular fluid of passive smokers in *in vitro* fertilization therapy. *Fertil. Steril.*, **66**, 614–619.

Zenzes, M.T., Libertad, A. P. and Bielecki, R. (1997) Immunodetection of cotinine protein in granulosa-lutein cells of women exposed to cigarette smoke. *Fertil. Steril.*, **68**, 76–82.