

The effect of female tobacco smoking on IVF outcomes

K.P.Wright^{1,5}, J.R.Trimarchi², J.Allsworth³ and D.Keefe⁴

¹Department of Obstetrics and Gynecology, ²In Vitro Fertilization Laboratory, ³Center for Gerontology and Health Care Research, Women and Infants Hospital, Brown Medical School, Providence, RI and ⁴Department of Obstetrics and Gynecology, University of South Florida, FL, USA

⁵To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Fletcher Allen Health Care, University of Vermont, 111 Colchester Ave, Burlington, VT 05401, USA. E-mail: Kristen.Wright@vtmednet.org

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 (E₂) 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 (E₂) levels, suggesting that smoking negatively impacts ovarian function

(Van Voorhis *et al.*, 1992); however, others demonstrate no difference in E₂ 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 ratio 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 E₂ 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 E₂ 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 E₂ 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 1. Descriptive characteristics by smoking status

| | Never smoked (%) | Currently smokes (%) | Smoked in past (%) | <i>P</i> 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/m² 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 E₂ 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 E₂ 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 <i>n</i> | Risk (preg/ <i>n</i>) | 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 |

^aToo 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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 \pm 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

We thank Sherry Weitzen, PhD, for her assistance in designing the study and calculating our initial power analysis.

References

- Augood C, Duckitt K and Templeton AA (1998) Smoking and female infertility: a systematic review and meta-analysis. *Hum Reprod* 13,1532–1539.
- Baird D and Wilcox AJ (1985) Cigarette smoking associated with delayed conception. *JAMA* 253,2979–2983.
- Campagna C, Sirard MA, Ayotte P and Bailey JL (2001) Impaired maturation, fertilization, and embryonic development of porcine oocytes following exposure to an environmentally relevant organochlorine mixture. *Biol Reprod* 65,554–560.
- Centers for Disease Control and Prevention (2004) Smoking during pregnancy—United States, 1990–2002. *MMWR Morb Mortal Wkly Rep* 8,911–915.
- Clarke EA, Morgan RW and Newman AM (1982) Smoking as a risk factor in cancer of the cervix: additional evidence from a case-control study. *Am J Epidemiol* 115,59–66.
- Elenbogen A, Lipitz S, Maschiach S, Dor J, Levran D and Ben-Rafael Z (1991) The effect of smoking on the outcome of in vitro fertilization-embryo transfer. *Hum Reprod* 6,242–244.
- Ellenbogen A (2004) The effect of male and female smoking on the outcome of in vitro fertilization-embryo transfer program. *Fertil Steril* 82,S131.
- Franklin W, Lowell FC, Michaelson AL and Schiller IW (1956) Chronic obstructive pulmonary emphysema; a disease of smokers. *Ann Intern Med* 45,268–274.
- Hammoud AO, Bujold E, Sorokin Y, Schild C, Krapp M and Baumann P (2005) Smoking in pregnancy revisited: findings from a large population-based study. *Am J Obstet Gynecol* 192,1862–1863.
- Harlap S and Baras M (1984) Conception-waits in fertile women after stopping contraceptives. *Int J Fertil* 29,73–80.
- Harrison KL, Breen TM and Hennessey JF (1990) The effect of patient smoking habit on the outcome of IVF and GIFT treatment. *Aust N Z J Obstet Gynaecol* 30,340–343.
- Hughes EG and Brennan B (1996) Does cigarette smoking impair natural or assisted fecundity? *Fertil Steril* 66,679–689.
- Hughes EG, Yeo J, Claman P, YoungLai EV, Sagle MA, Daya S and Collins JA (1994) Cigarette smoking and the outcomes of in vitro fertilization: measurement of effect size and levels of action. *Fertil Steril* 62,807–814.
- Hull MG, North K, Taylor HM, Farrow A and Ford CW (2000) Delayed conception and active and passive smoking. *Fertil Steril* 74,725–733.
- Jenkins CD, Rosenman RH and Zyzanski SJ (1968) Cigarette smoking. Its relationship to coronary heart disease and related risk factors in the Western Collaborative Group Study. *Circulation* 38,1140–1155.
- Jick H and Porter J (1977) Relation between smoking and age of natural menopause. Report from the Boston Collaborative Drug Surveillance Program, Boston University Medical Center. *Lancet* 25,1354–1355.
- Kannel WB, McGee D and Gordon T (1976) A general cardiovascular risk profile: the Framingham Study. *Am J Cardiol* 38,46–51.
- Kapawa A, Giannakis D, Tsoukanelis K, Kanakas N, Baltogiannis E, Agapitos D, Loutradis D, Miyagawa I and Sofikitis N (2004) Effects of paternal cigarette smoking on testicular function, sperm fertilizing capacity, embryonic development, and blastocyst capacity for implantation in rats. *Andrologia* 36,57–68.
- Kyrklund-Blomberg NB, Granath F and Cnattingius S (2005) Maternal smoking and causes of very preterm birth. *Acta Obstet Gynecol Scand* 84,572–577.
- Laurent SL, Thompason SJ, Addy C, Garrison CZ and Moore EE (1992) An epidemiologic study of smoking and primary infertility in women. *Fertil Steril* 57,565–572.
- McNutt LA, Wu C, Xue X and Hafner JP (2003) Estimating the Relative Risk in Cohort Studies and Clinical Trials of Common Outcomes. *Am J Epidemiol* 157,940–943.
- Neal MS, Hughes E, Holloway A and Foster W (2005) Sidestream smoking is equally as damaging as mainstream smoking on IVF outcomes. *Hum Reprod* 20,2531–2535.
- Pattinson HA, Taylor PJ and Pattinson MH (1991) The effect of cigarette smoking on ovarian function and early pregnancy outcome of in vitro fertilization treatment. *Fertil Steril* 55,780–783.
- Rosevear SK, Holt DW, Lee TD, Ford WC, Wardle PG and Hull MG (1992) Smoking and decreased fertilization rates in vitro. *Lancet* 340,1195–1196.
- Rowlands DJ, McDermott A and Hutt MGR (1992) Smoking and decreased fertilization rates in vitro. *Lancet* 340,1409–1410.
- Seltzer V (2003) Smoking as a risk factor in the health of women. *Int J Gynaecol Obstet* 82,393–397.
- Sofikitis N, Miyagawa I, Dimitriadis D, Zavos P, Sikka S and Hellstrom W (1995) Effects of smoking on testicular function, semen quality and sperm fertilizing capacity. *J Urol* 154,1030–1034.
- Sterzik K, Strehler E, De Santo M, Trumpp N, Abt M, Rosenbusch B and Schneider A (1996) Influence of smoking on fertility in women attending an in vitro fertilization program. *Fertil Steril* 65,810–814.
- Terriou P, Sapin C, Hans E, Spach JL, Roulier R (2001) Embryo score is a better predictor of pregnancy than the number of transferred embryos or female age. *Fertil Steril* 75,525–531.
- Trapp M, Baukloh V, Bohnet HG and Heeschen W (1984) Pollutants in human follicular fluid. *Fertil Steril* 42,146–148.
- Trapp M, Kemeter P and Feichtinger W (1986) Smoking and in-vitro fertilization. *Hum Reprod* 1,357–358.
- Van Voorhis BJ, Syrop CH, Hammit DG, Dunn MS and Snyder GD (1992) Effects of smoking on ovulation induction for assisted reproductive techniques. *Fertil Steril* 58,981–985.
- Wainright RL (1983) Change in observed birth weight associated with change in maternal cigarette smoking. *Am J Epidemiol* 117,668–675.
- Watson WL and Conte AJ (1954) Smoking and lung cancer. *Cancer* 7,245–249.
- Younglai EV, Foster WG, Hughes EG, Trim K and Jarrell JF (2002) Levels of environmental contaminants in human follicular fluid, serum, and seminal plasma of couples undergoing in vitro fertilization. *Arch Environ Contam Toxicol* 43,121–126.
- Zenzes MT (2000) Smoking and reproduction: gene damage to human gametes and embryos. *Hum Reprod Update* 6,122–131.
- Zenzes MT, Reed TE, Wang P and Klein J (1996) Cotinine, a major metabolite of nicotine, is detectable in follicular fluids of passive smokers in in vitro fertilization therapy. *Fertil Steril* 66,614–619.

Submitted on July 6, 2005; resubmitted on February 1, 2006, May 21, 2006; accepted on May 25, 2006