

Sidestream smoking is equally as damaging as mainstream smoking on IVF outcomes

Michael S.Neal^{1,2,3}, Edward G.Hughes^{1,2}, Alison C.Holloway¹ and Warren G.Foster^{1,2}

¹Reproductive Biology Division, Department of Obstetrics and Gynecology, McMaster University, 1200 Main Street West, Hamilton, Ontario, L8N 3Z5 and ²Centre for Reproductive Care, Hamilton Health Sciences, 690 Main Street West, Hamilton, Ontario, L8S 1A4, Canada

³To whom correspondence should be addressed at: Centre for Reproductive Care, Hamilton Health Sciences, 690 Main Street West, Hamilton, Ontario, Canada, L8S 1A4. E-mail: nealm@hhsc.ca

BACKGROUND: Cigarette smoking (CS) is a widely recognized health hazard, yet it remains prevalent in society and the effects of environmental tobacco smoke exposure on fertility are unknown. Our objective was to measure the effects of CS on the fertility of mainstream (MS) or sidestream (SS) smoke-exposed women compared to their non-smoking (NS) counterparts. **METHODS:** This retrospective study investigated 225 female patients undergoing IVF ($n = 97$) or ICSI ($n = 128$). Patients were grouped based on their smoking status for comparison. This included: 39 MS (18 IVF and 21 ICSI); 40 SS (16 IVF and 24 ICSI); and 146 NS (63 IVF and 83 ICSI) women. Fertility treatment outcomes including embryo quality, implantation and pregnancy rate were measured. **RESULTS:** No difference in embryo quality between the three groups was observed. However, there was a significant difference in implantation rate (MS = 12.0%, SS = 12.6%, and NS = 25.0%) and pregnancy rate (MS = 19.4%, SS = 20.0%, and NS = 48.3%) per embryo transfer. **CONCLUSIONS:** Despite similar embryo quality there was a striking difference in implantation and pregnancy rates of MS and SS smokers when compared with NS. Our data demonstrate that the effects of SS smoking are equally as damaging as MS smoke on fertility.

Key words: infertility/ovarian function/smoking/toxicants

Introduction

Despite the fact that tobacco smoking is a widely recognized health hazard and a major cause of preventable mortality, smoking remains prevalent in our society. While there has been a decrease in the incidence of smoking in the general population, the percentage of female smokers has increased considerably (Health Canada, 2003). What is even more troubling is the incidence of smoking among young women. A recent survey of high school students in southwestern Ontario revealed that 36.2% of teenage girls smoke (Cohen *et al.*, 2003) and a study from the UK reports that, at the age of 15 years, 33% of girls are regular smokers (Augood *et al.*, 1998).

The reproductive health effects of cigarette smoke exposure for women are far-reaching (Seltzer, 2003). There is a strong association between cigarette smoking and reduced fertility, and earlier mean age of menopause (Jick and Porter, 1977), suggesting that smoking impairs oocyte function, viability, and depletes ovarian reserves (Zenzes, 2000). Epidemiological studies of spontaneous conception confirm that 38% of non-smokers conceive in their first cycle compared with 28% of smokers, with smokers being 3.4 times more likely to take >1 year to conceive compared to their non-smoking counterparts (Baird and Wilcox, 1985).

In addition, Howe *et al.* (1985) reported that 10.7% of women who smoke >10 cigarettes/day, compared to 5.4% of non-smokers, are involuntarily childless 5 years after stopping contraception. Although cigarette smoke is a recognized reproductive hazard the impact of passive or sidestream smoke exposure on female fertility is unknown. Therefore, the objective of this study was to investigate the adverse effects of cigarette smoke exposure on fertility and ovarian function by comparing stimulation parameters and outcomes of mainstream (MS), sidestream (SS), and non-smoking (NS) patients undergoing IVF procedures.

Materials and methods

Patients and methods

Approval was obtained from the Centre for Reproductive Care (CRC) Research committee for this study. This was a retrospective analysis of the outcomes of 225 patients (97 IVF and 128 ICSI) being treated at the CRC for infertility between January 1, 2003 and March 31, 2004. Smoking status and the average number of cigarettes consumed daily was obtained from the patients' preliminary fertility assessment form completed on the first visit to the clinic. Alcohol consumption in our study population was unremarkable. These data were routinely collected on all patients using a standardized history form. Patients were classified into one of three groups:

(1) mainstream smoke (MS, $n = 39$: 18 IVF and 21 ICSI); (2) sidestream smoke (SS, $n = 40$: 16 IVF and 24 ICSI); or (3) non-smoking (NS, $n = 146$: 63 IVF and 83 ICSI). Mainstream smoke was defined as the smoke that is inhaled by the smoker; while patients with sidestream smoke exposure were defined as those women self-identifying that they live with a partner that regularly smokes. Non-smoking patients were defined as someone not exposed in either of the previous two categories. Former smokers (ceased smoking >6 months prior to treatment) were considered with non-smokers since several studies have shown that the fertility of ex-smokers resembles that of non-smokers, rather than that of current smokers (Baird and Wilcox, 1985; Howe *et al.*, 1985; Phipps *et al.*, 1987).

IVF stimulation and outcomes

Female patients each received subcutaneous injections of GnRH analogue, leuprolide acetate (Lupron; Abbott, Toronto, ON, Canada) 1 mg daily, from day 21 of the previous menstrual cycle for pituitary down-regulation. Recombinant rFSH (Gonal F; Serono, Oakville, ON, Canada) was used for all patients in this study and was initiated after the onset of menses after ovarian suppression was confirmed by a serum estradiol of <150 pmol/ml and no ovarian cysts were observed upon ultrasound examination. Dosage of rFSH was adjusted based on the rise in estradiol levels measured by radioimmunoassay, and the numbers of growing follicles were tracked by transvaginal ultrasound. Ovulation was triggered, when at least three follicles were ≥ 18 mm in diameter, by administering 10 000 IU i.m. of hCG (Profasi; Serono). Oocytes were retrieved using transvaginal ultrasound-guided aspiration 34–36 h after the hCG injection. Antibiotics were routinely administered intravenously to patients with blocked tubes only at the time of retrieval (Paavonen and Eggert-Kruse, 1999). Medication use by patients in all three groups was assessed and there was no difference in the types or amounts of medications used between the groups.

Semen samples were provided by the male partner and processed by standard swim-up procedures. Insemination for conventional IVF or ICSI was performed 4–6 h after oocyte retrieval. Oocytes and embryos were cultured in 60 μ l drops of human tubal fluid medium (HTF; Irvine Scientific, Santa Ana, CA, USA) supplemented with 10% synthetic serum substitute (SSS; Irvine Scientific). Fertilization was determined by the presence of two pronuclei 16–18 h after insemination or ICSI. Fertilization rate was calculated as the number of zygotes divided by the total number of oocytes retrieved for each patient.

The stimulation parameters, number of oocytes retrieved, fertilization, meiotic maturity based on the presence of the first polar body at the time of ICSI, embryos transferred, embryos frozen, cumulative embryo score (CES), implantation and pregnancy rates were compared between the three groups. CES was calculated as the product of blastomere number and embryo grade based on percentage fragmentation (descending scale of 5 to 1) at the 48 and 72 h period of assessment. Higher CES indicates a better morphological appearance of the embryo (Joesbury *et al.*, 1998; Terriou *et al.*, 2001). Based on our clinic policy, a maximum of two embryos were transferred to women aged <34 years of age, and a maximum of three embryos to those women ≥ 34 years of age, on day 3 of culture. Assisted hatching was not performed on any of the transferred embryos. Three patients in the MS group did not have adequate embryos for transfer, and one patient in the NS group had all of her embryos frozen due to severe ovarian hyperstimulation syndrome and thus their data was used only for stimulation and embryo development outcomes and was excluded from the pregnancy analysis since no embryo transfer occurred. A positive pregnancy test was

characterized by an elevated β -hCG 14 days after transfer, and confirmed by a doubling in value 3 days later. Clinical pregnancy was confirmed by a positive fetal heartbeat by ultrasound, 6.5 weeks after a positive pregnancy test (β -hCG). The number of fetal sacs with a positive heartbeat divided by the total number of embryos transferred was used to calculate the implantation rate.

Statistical analysis

Values are expressed as mean \pm SD where applicable. Rates were expressed as percentages and the significance of differences between rates were determined using the classical χ^2 -test. Data were checked for homogeneity of variance and normal distribution before analysis by one-way analysis of variance. Differences between group means were determined by Dunnett's method. Statistical analysis was performed using SigmaStat (SPSS, Chicago, IL, USA). For all statistical procedures performed, $P < 0.05$ was considered significant.

Results

Smoke exposure

Women in the MS category consumed a mean (\pm SEM) of 11.0 ± 1.2 cigarettes/day and their partners reported smoking a mean of 10.7 ± 2.0 cigarettes/day. Of the male partners of MS cigarette smoke-exposed women, eight were non-smokers, 12 smoked <12 cigarettes/day, and 15 smoked >12 cigarettes/day. Four male partners were reported to be smokers but the daily consumption was not available. Male partners of the women in the SS group smoked 10.8 ± 1.0 cigarettes/day. Of this group, 20 partners smoked <12 cigarettes/day whereas 16 consumed >12 cigarettes/day. Frequency of smoking and number of cigarettes smoked was unavailable for four of the male partners. When the pregnancy outcomes were compared based on male smoking habits, there was no difference in the number of cigarettes/day consumed by the male partner between the pregnant and non-pregnant groups in both the MS and SS categories. Furthermore, there was no difference in the pregnancy rate of the MS group when the partners' smoking status was considered (NS versus smoker), thus indicating that cigarette smoke exposure in the male partner was not solely responsible for the negative impact on pregnancy.

Clinical outcomes

There was no difference in the age of patients, units of FSH used for stimulation, estradiol level on the day of hCG injection, number of oocytes retrieved, nuclear maturity, number of embryos transferred, and embryos available for freezing between the three groups (Table I). Fertilization rates were similar in the MS (57%), SS (58%) and NS (63%) groups. Despite no difference in embryo quality, assessed by CES at 48, 72 h, and of the embryos being included for transfer (Figure 1), there was a significant difference ($P < 0.001$) in pregnancy rate per embryo transfer between the MS (19.4%), SS (20.0%) and NS (48.3%) groups. Implantation rate was also different ($P < 0.01$) between the MS (12.0%), SS (12.6%) and NS (25.0%) groups.

Table I. Clinical summary, mean \pm SD by smoking status

Variable	Mainstream (n = 39)	Sidestream (n = 40)	Non-smoking (n = 146)
Age (years)	33.56 \pm 3.84	33.45 \pm 3.35	34.57 \pm 3.95
Ampoules for stimulation	29.18 \pm 14.25	30.23 \pm 16.25	32.40 \pm 14.88
Estradiol on day of hCG (pmol/L)	7142 \pm 3388	7921 \pm 4179	7316 \pm 3721
Oocytes retrieved	11.26 \pm 5.32	11.55 \pm 5.64	11.09 \pm 5.70
Nuclear maturity (%) ^a	80.0 \pm 19.0	78.0 \pm 18.0	77.0 \pm 17.0
Embryos transferred	1.92 \pm 0.81	2.18 \pm 0.50	2.32 \pm 0.87
Embryos frozen	1.82 \pm 2.91	1.58 \pm 2.57	1.53 \pm 2.02

^aNuclear maturity was only measured in ICSI procedures.

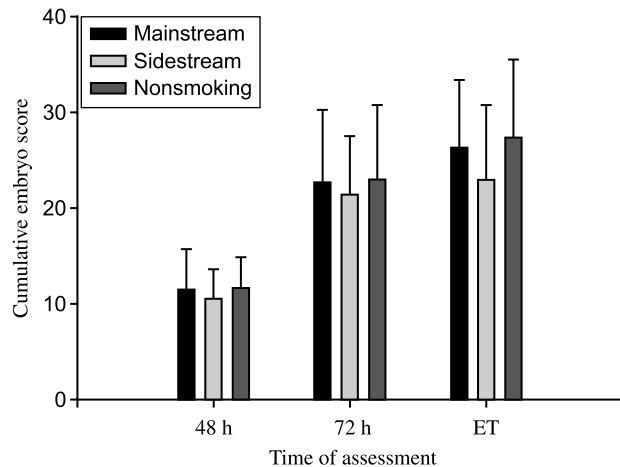


Figure 1. Comparison of Cumulative Embryo score (CES) between Mainstream (MS), Sidestream (SS) and Nonsmoking patients' embryos at 48 h, 72 h, and transfer.

Discussion

The objective of the present study was to investigate the effect of side-stream cigarette smoke exposure compared to mainstream exposure and no exposure (non-smoking women) on fertility in female patients attending the Centre for Reproductive Care. The most striking finding is that despite no difference in morphological appearance and development rate of preimplantation embryos from MS, SS and NS women, there is a significant decrease in the ability of embryos from MS and SS smokers to implant and/or maintain a pregnancy when compared to their NS counterparts. Our data are consistent with previous studies that demonstrate reduced fertility in female smokers (Pattinson *et al.*, 1991; Rosevear *et al.*, 1992; Rowlands *et al.*, 1992; Hughes *et al.*, 1994; Seltzer, 2003) and expands the literature by demonstrating that the reproductive consequences of side-stream smoking are as great as those seen in mainstream smokers.

In the present study, no evidence of either a dose-response (SS versus MS exposure) or an additive effect (both partners were smokers) of cigarette smoke exposure on pregnancy could be found. The women in our study were divided into groups of MS, SS and NS on the basis of self-reported

smoking habits for themselves and their partners, thus raising the potential for misclassification errors. In prospective studies, smoking status can be confirmed biochemically by measuring cotinine, a metabolite of nicotine, as cotinine concentrations correlate with the number of cigarettes smoked (Zenzes *et al.*, 1996). However, as the present study was retrospective in nature we did not have the benefit of confirming smoking status or testing for potential dose-related effects. Nevertheless, we propose that SS cigarette smoke exposure is as hazardous as that of MS smoke. Support for this proposal is derived from the observation that the polycyclic aromatic hydrocarbons (PAH) produced by the inefficient combustion from the tip of the smoldering cigarette resulted in a 10-fold higher concentration of PAH in SS compared with MS smoke (Lodovici *et al.*, 2004). Of the contaminants present in cigarette smoke, the PAH have been shown to induce a broad range of adverse health effects on reproductive outcomes in rodent and human studies (Borman *et al.*, 2000; Zenzes, 2000). Therefore, it is not surprising that the toxic effect of SS exposure, measured by reduced fecundity, is similar to that seen in women who smoke.

The mechanism underlying the reduced fertility success in women exposed to cigarette smoke is unknown. Toxicants can impair reproduction by acting in the male, female, or both (Stillman *et al.*, 1986). Several studies have focused on the effect of paternal cigarette smoke exposure on spermatogenesis (Sofikitis *et al.*, 1995; Yamamoto *et al.*, 1998), sperm function and early embryonic development (Kapawa *et al.*, 2004). Sofikitis *et al.* (2000) demonstrated that cotinine (a metabolite of nicotine) concentrations similar to that seen in heavy smokers (>400 ng/ml) reduced sperm performance with respect to *in vitro* tests (including: hyperosmotic swelling test, sperm penetration assay, % motility and hyperactivation), but did not affect fertilization of hamster ova when ICSI was employed. Results from our study did not reveal any reduction in sperm function as indicated by similar IVF fertilization rates of men exposed to mainstream or sidestream smoke compared with non-smokers. We propose that in our study cotinine levels are unlikely to have achieved high enough levels to elicit a sperm effect since none of our patients were considered heavy smokers. Moreover, ICSI fertilization rates were similar, which is supported by the work of Sofikitis *et al.* (2000). Paternal smoking results in a significantly higher percentage of sperm with DNA abnormalities in the semen samples of smokers compared to non-smokers (Sofikitis *et al.*, 1995). Sperm with a high percentage of DNA fragmentation are often found in cases of repeated IVF failures (Sakkas *et al.*, 2004; Tesarik *et al.*, 2004). Adverse paternal effects can appear as early as the pronuclear zygote stage or later in preimplantation development (Tesarik *et al.*, 2002). Since embryos were transferred on day 3 in this study it is not clear whether a late paternal effect could influence blastocyst development if the culture period was extended an additional 2 days. However, our results demonstrate that despite no difference in morphological appearance and development rate of preimplantation embryos from MS, SS and NS women, there is a significant decrease in the ability of embryos from MS and SS smokers

to implant and/or maintain a pregnancy when compared to their NS counterparts. Therefore, we propose that implantation and embryogenesis are the most sensitive stages of development for the adverse effects of cigarette smoke. This may occur as a result of a late paternal effect (Sakkas *et al.*, 2004; Tesarik *et al.*, 2004) or might be due to an alteration of ovarian function and/or impaired oocyte competence from exposure to toxic compounds. Cigarette smoke may induce changes in oocyte quality through the deleterious effect of ovarian toxicants present in the follicular fluid. Zenzes (2000) observed the formation of DNA adducts in the ovarian cells and embryos of smokers, indicating a potential risk of genetic damage such as spindle disturbances, or structural and numerical chromosomal aberrations. These types of subtle disruptions to the genome of the gamete or zygote may allow for normal-appearing preimplantation embryo development as we observed (Figure 1) but may be lethal at the peri-implantation stage leading to reduced implantation rates. Also, PAH such as benzo-[a]-pyrene have been shown to markedly decrease proliferation, cell attachment and trophoblast invasion (Charles *et al.*, 2000) which may account for the poor implantation rates and early pregnancy loss observed in our study. Poor IVF outcomes may also be attributed in part to defects in the sperm as a result of exposure to smoke-related toxicants (Joesbury *et al.*, 1998). However, our data reveal no difference in pregnancy rate when the smoking habit of the male partner is considered. Therefore, our data suggest that exposure to toxicants contained in cigarette smoke, whether from MS or SS exposure, adversely affects implantation success and maintenance of pregnancy. Fortunately, for women who seek medical attention, a recent randomized controlled trial found that a simple explanation of the reproductive consequences of cigarette smoking was as effective as a structured intervention in achieving smoking cessation (Hughes *et al.*, 2000).

Cigarette smoke toxicants may induce toxic effects and compromise fertility through several direct or indirect mechanisms. However, in the present study there was no evidence of an adverse effect of cigarette smoke exposure on ovarian function as shown by similar ovarian stimulation response and serum estradiol levels between the MS, SS and NS groups. Our findings are in contrast to prior studies in which decreased serum estradiol levels of female smokers compared to non-smokers were reported (Van Voorhis *et al.*, 1992). However, Van Voorhis *et al.* (1992) did note that there was a similar estradiol concentration per follicle, so our observation may be a reflection of number of oocytes recruited by our stimulation protocol. Other studies have reported similar outcomes in stimulation parameters such as estradiol level and oocytes retrieved between smoking and non-smoking women undergoing infertility treatment (Hughes *et al.*, 1994; Sterzik *et al.*, 1996). Furthermore, cigarette smoke has been shown to affect critical control mechanisms of intra-follicular processes such as oocyte maturation and cumulus expansion (Vrsanska *et al.*, 2003). Earlier studies have shown that aqueous tobacco smoke extracts inhibit granulosa cell aromatase activity (Barbieri *et al.*, 1986). This, taken together with our recent finding that granulosa cell aromatase activity is

related to pregnancy potential (Neal *et al.*, 2004), suggests that toxicants from cigarette smoke may be interfering with this process of estrogen production by blocking aromatase activity. Inhibition of granulosa-luteal cell function may lead to corpus luteal deficiency, which could be one underlying mechanism to explain the higher rate of early pregnancy loss in women exposed to smoke (Shiverick and Salafia, 1999).

Results of the present study demonstrate that the clinical pregnancy rate in women exposed to side-stream smoke is similar to that of women with mainstream cigarette smoke exposure and significantly lower than for non-smokers. As there was no difference in pregnancy rate when the smoking status of the partner was considered, we conclude that the reproductive consequences of SS smoking are as great as those observed in active smokers. Although important, these results will need to be confirmed in a prospective study with more objective measures of cigarette smoke exposure. Furthermore, while there have been numerous studies of the effects of cigarette smoke exposure on the male gamete, our results suggest that similar investigations should be extended to the female.

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 DD and Wilcox AJ (1985) Cigarette smoking associated with delayed conception. *J Am Med Assoc* 253,2979–2983.
- Barbieri RL, McShane PM and Ryan KJ (1986) Constituents of cigarette smoke inhibit human granulosa cell aromatase. *Fertil Steril* 46,232–236.
- Borman SM, Christian PJ, Sipes IG and Hoyer PB (2000) Ovotoxicity in female Fischer rats and B6 mice induced by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index. *Toxicol Appl Pharmacol* 167,191–198.
- Charles GD, Bartels MJ, Zacharewski TR, Gollapudi BB, Freshour NL and Carney EW (2000) Activity of benzo[a]pyrene and its hydroxylated metabolites in a estrogen receptor-alpha reporter gene assay. *Toxicol Sci* 55,320–326.
- Cohen B, Evers S, Manske S, Bercovitz K and Edward HG (2003) Smoking, physical activity and breakfast consumption among secondary school students in a southwestern Ontario community. *Can J Publ Hlth* 94,41–44.
- Health Canada (2003) Canadian Tobacco Use Monitoring Survey E-mail: http://www.hc-hcgc.ca/hecshec/tobacco/research/ctums/2003/supplementary_annual_2003.html.
- Howe G, Westhoff C, Vessey M and Yeates D (1985) Effects of age, cigarette smoking and other factors on fertility: findings in a large prospective study. *Br Med J (Clin Res Ed)* 290,1697–1700.
- Hughes EG, Lamont DA, Beecroft ML, Wilson DMC, Brennan BG and Rice SC (2000) Randomized trial of a "state-of-change" oriented smoking cessation intervention in infertile and pregnant women. *Fertil Steril* 74, 498–503.
- 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.
- 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* 1,1354–1355.
- Joesbury KA, Edirisinghe WR, Phillips MR and Yovich JL (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.
- Kapawa A, Giannakis D, Tsoukanelis K, Kanakas N, Baltogiannis D, Agapitos E, 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.

- Lodovici M, Akpan V, Evangelisti C and Dolara P (2004) Sidestream tobacco smoke as the main predictor of exposure to polycyclic aromatic hydrocarbons. *J Appl Toxicol* 24,277–281.
- Neal MS, Younglai EV, Holloway AC and Foster WG (2004) Aromatase activity in granulosa cells as a predictor of pregnancy potential. *Int Congr Series* 1271,139–142.
- Paavonen J and Eggert-Kruse W (1999) Chlamydia trachomatis: impact on human reproduction. *Hum Reprod Update* 5,433–447.
- 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.
- Phipps WR, Cramer DW, Schiff I, Belisle S, Stillman R, Albrecht B, Gibson M, Berger MJ and Wilson E (1987) The association between smoking and female infertility as influenced by cause of the infertility. *Fertil Steril* 48,377–382.
- Rosevear SK, Holt DW, Lee TD, Ford WC, Wardle PG and Hull MG (1992) Smoking and decreased fertilisation rates in vitro. *Lancet* 340,1195–1196.
- Rowlands DJ, McDermott A and Hull MG (1992) Smoking and decreased fertilisation rates in vitro. *Lancet* 340,1409–1410.
- Sakkas D, D'Arcy Y, Percival G, Sinclair L, Afnan M and Sharif K (2004) Use of the egg-share model to investigate the paternal influence on fertilization and embryo development after in vitro fertilization and intracytoplasmic sperm injection. *Fertil Steril* 82,74–79.
- Seltzer V (2003) Smoking as a risk factor in the health of women. *Int J Gynaecol Obstet* 82,393–397.
- Shiverick KT and Salafia C (1999) Cigarette smoking and pregnancy. I: ovarian, uterine and placental effects. *Placenta* 20,265–272.
- Sofikitis N, Takenaka M, Kanakas N, Papdopoulos H, Yamamoto Y, Drakakis P and Miyagawa I (2000) Effects of cotinine on sperm motility, membrane function, and fertilizing capacity in vitro. *Urol Res* 28(6): 370–375.
- 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.
- Stillman RJ, Rosenberg MJ and Sachs BP (1986) Smoking and reproduction. *Fertil Steril* 46,545–566.
- Terriou P, Sapin C, Giorgetti C, Hans E, Spach JL and 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.
- Tesarik J, Mendoza C and Greco E (2002) Paternal effects acting during the first cell cycle of human preimplantation development after ICSI. *Hum Reprod* 17,184–189.
- Tesarik J, Greco E and Mendoza C (2004) Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. *Hum Reprod* 19,611–615.
- Van Voorhis BJ, Syrop CH, Hammitt DG, Dunn MS and Snyder GD (1992) Effects of smoking on ovulation induction for assisted reproductive techniques. *Fertil Steril* 58,981–985.
- Vrsanska S, Nagyova E, Mlynarcikova A, Fickova M and Kolena J (2003) Components of cigarette smoke inhibit expansion of oocyte-cumulus complexes from porcine follicles. *Physiol Res* 52,383–387.
- Yamamoto Y, Isoyama E, Sofikitis N and Miyagawa I (1998) Effects of smoking on testicular function and fertilizing potential in rats. *Urol Res* 26,45–48.
- Zenzen MT (2000) Smoking and reproduction: gene damage to human gametes and embryos. *Hum Reprod Update* 6,122–131.
- Zenzen 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 February 1, 2005; resubmitted on March 18, 2005; accepted on April 20, 2005