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Effects of cigarette smoking upon clinical outcomes of assisted reproduction: a meta-analysis

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BACKGROUND: The aim of this meta-analysis was to investigate whether any difference exists in success rate of clinical outcomes of assisted reproductive technologies (ART) between women who actively smoke cigarettes at the time of treatment and those who do not.

METHODS: An intensive computerized search was conducted on published literature from eight databases, using search terms related to smoking, assisted reproduction and outcome measures. Eligible studies compared outcomes of ART between cigarette smoking patients and a control group of non-smoking patients and reported on live birth rate per cycle, clinical pregnancy rate per cycle, ectopic pregnancy rate per pregnancy or spontaneous miscarriage rate per pregnancy, and 21 studies were included in the meta-analyses. Pooled odds ratios (OR) and 95% confidence intervals (CI) were calculated for the data, and statistical heterogeneity was tested for using χ^2 and I^2 values. A systematic review examined the effect of smoking upon fertilization rates across 17 studies.

RESULTS: Smoking patients demonstrated significantly lower odds of live birth per cycle (OR 0.54, 95% CI 0.30–0.99), significantly lower odds of clinical pregnancy per cycle (OR 0.56, 95% CI 0.43–0.73), significantly higher odds of spontaneous miscarriage (OR 2.65, 95% CI 1.33–5.30) and significantly higher odds of ectopic pregnancy (OR 15.69, 95% CI 2.87–85.76). A systematic literature review revealed that fertilization rates were not significantly different between smoking and non-smoking groups in most studies.

CONCLUSIONS: This meta-analysis provides compelling evidence for a significant negative effect of cigarette smoking upon clinical outcomes of ART and should be presented to infertility patients who smoke cigarettes in order to optimize success rates.

Key words: assisted reproduction / cigarette / IVF / meta-analysis / smoking

Introduction

Cigarette smoking is well documented to be a significant health risk and major cause of premature mortality, yet it remains a prevalent lifestyle habit in today's society.

Although the negative effects of cigarette smoking on general health are well known, the effects of smoking on assisted reproduction technologies (ART) are less well documented, and conflicting reports continue to be published regarding the effect. While many studies suggest that cigarette smoking has a negative influence upon outcomes of ART, the exact mechanism of this effect is poorly understood. A recent systematic review of the evidence revealed that smoking may affect rates of oocyte retrieval, IVF, embryo transfer, pregnancy and live birth delivery (Klonoff-Cohen, 2005).

Contrary to these results, not all studies have demonstrated statistically significant negative effects of smoking on ART outcome (Trapp *et al.*, 1986; Hughes *et al.*, 1994; Sharara *et al.*, 1994; Sterzik *et al.*, 1996; Weigert *et al.*, 1999; Wright *et al.*, 2006).

Research has shown that providing ART patients with information detailing the negative impact of cigarette smoking upon their fertility can be highly effective in achieving smoking cessation (Hughes *et al.*, 2000). Therefore, if a significant effect of smoking upon clinical outcomes of ART does in fact exist, clinicians can make a measurable impact upon ART success by sharing this data with their patients.

Two previous meta-analyses have investigated the effect of cigarette smoking on clinical pregnancy rates for IVF (Feichtinger *et al.*, 1997; Augood *et al.*, 1998). The relatively small number of studies pooled and the large body of evidence that has been published since this time means that there is a need for an up-to-date meta-analysis to be completed to provide clear evidence on any effects of cigarette smoking on ART.

The aim of this meta-analysis is to combine the results from the growing body of evidence regarding ART success rates in smoking and non-smoking patients.

Materials and Methods

Criteria for including studies for this review

Types of studies

All human studies reporting outcomes of ART compared female cigarette smoking patients with a control group of non-smoking patients who report on a minimum of one the following outcomes:

(i) Live birth rate per cycle
(ii) Clinical pregnancy rate per cycle
(iii) Ectopic pregnancy rate per pregnancy
(iv) Spontaneous miscarriage rate per pregnancy
(v) Fertilization rate per cycle

Due to the nature of the research question, we did not expect to retrieve any randomized control trials from the literature search, and accordingly only cohort and case–control studies were included in this review. Studies that only investigated the effect of passive cigarette smoking were excluded. For the present review, only published material was included to ensure quality of studies and results.

Types of intervention

ART included in the review were IVF, intracytoplasmic sperm injection (ICSI), gamete intrafallopian transfer (GIFT), and zygote intrafallopian transfer (ZIFT). Studies reporting results for spontaneous pregnancy or intra-uterine insemination were excluded.

Types of participant

Study inclusion was limited to those reporting outcomes of assisted reproduction in active cigarette smokers versus non-smoking patients at the time of treatment.

Where studies differentiated between non-smokers and ex-smokers, only the results for non-smokers were used. Although evidence has been published suggesting that the fertility of an ex-smoker resembles that of a non-smoker (Phipps *et al.*, 1987), there is no standardized definition of an 'ex-smoker' and publications rarely stated their definition in terms of the time since stopping smoking. In an attempt to eliminate this likely cause of heterogeneity between studies, we did not include ex-smokers in the meta-analysis.

Where studies reported outcomes after oocyte donation, the smoking status of the recipient was used for analysis rather than that of the donor, as the outcomes measured were following successful embryo transfer.

Types of outcome measure

The outcome measures for this meta-analysis were:

(i) Live birth rate: defined as the birth of one or more infants that show signs of life.
(ii) Clinical pregnancy rate: defined as the presence of a sonographically visible gestational sac in the uterus.
(iii) Spontaneous miscarriage rate: defined as loss of pregnancy up to 20 weeks gestation.
(iv) Ectopic pregnancy rate: defined as the presence of an extra-uterine pregnancy diagnosed by ultrasound or laparoscopy.
(v) Fertilization rate: defined as the proportion of oocytes fertilized.

Search strategy for identification of studies

An intensive computerized search was conducted on published literature from eight databases in May 2007. The databases searched (inclusive dates) were Medline (1950–2007), Embase (1980–2007), Cochrane library (1991–2007), PsycINFO (1806–2007), Science Citation Index (1900–2007), Social Science Citation Index (1900–2007), CINAHL (1982–2007) and Medline In-Process and Other Non-Indexed Citations. No date or language restrictions were used.

The search strategy used combinations of search terms related to smoking, assisted reproduction and outcome measures. Search terms used related to smoking were smoking, cigarette and tobacco. Terms relating to assisted reproduction were *in vitro* fertilization, IVF, assisted reproduction, assisted conception, artificial reproduction, fertility, infertility, subfertility, fecundity and subfecundity. Terms related to outcome measures were pregnancy rate, birth rate, ovarian reserve, ovulation induction, fertilization rate, conception rate, embryo quality, cycle cancellation rate, miscarriage rate and ectopic pregnancy rate.

Data extraction

The literature search (see Fig. 1) retrieved 356 references for possible inclusion in the meta-analysis. After reading the title and abstract where necessary of these references, 312 articles were excluded for not addressing the primary research question. A further 11 publications were excluded on this basis after retrieving the full article for further study. Of the remaining 33 publications identified as addressing the primary research question, a further 12 studies were excluded: four studies were not published in English and therefore did not allow their methodological quality to be fully evaluated (Hruba *et al.*, 1999; pa-Martynow *et al.*, 2005, 2006; Triopon *et al.*, 2006), three articles (Hughes *et al.*, 1992; Van Voorhis *et al.*, 1992; Crha *et al.*,

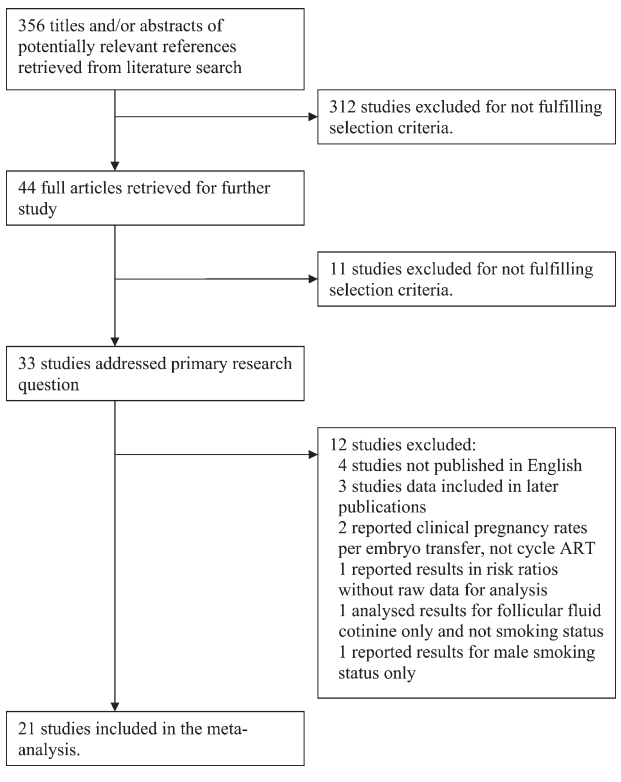

Figure 1 Flow chart of literature search and data extraction.

2000) presented data from the same cohort of patients published in other included studies (Hughes *et al*., 1994; Van Voorhis *et al*., 1996; Crha *et al*., 2001) and two studies reported clinical pregnancy rates per embryo transfer and not per cycle ART (Neal *et al*., 2005; Motejlek *et al*., 2006). One study could not be included due to reporting results in the form of risk ratios without raw data that could be pooled by meta-analysis (Klonoff-Cohen *et al*., 2001), one analysed results with regard to follicular fluid cotinine values alone and not smoking status (Zenzes *et al*., 1997), and the final study analysed results with regard to male partner smoking status only (Zitzmann *et al*., 2003).

The remaining 21 studies met all inclusion criteria for this meta-analysis. Bibliographies of relevant studies were hand searched but did not identify any other potentially relevant studies. The characteristics of studies included in the meta-analysis are summarized in Table I.

Statistical analysis

Dichotomous data were extracted from the individual studies. Revman software [Review Manager (RevMan), Version 4.2 for Windows, Copenhagen: the Nordic Cochrane Centre, The Cochrane Collaboration, 2003] was used to express these results as combined odds ratio (OR) with 95% confidence intervals (CI), and to combine these results for meta-analysis where primary outcomes were reported by two or more studies. Statistical heterogeneity between studies was identified by a chi-squared value greater than its degree-of-freedom, or an I^2 value >25%. Where this was the case, a random effects model was used for meta-analysis. A fixed effect method for meta-analysis was used to evaluate risk of ectopic pregnancy: in this case there was no significant evidence of heterogeneity.

We initially combined all studies together to calculate pooled OR for the outcomes regardless of the method of ART, and then performed a subgroup analysis based on method of ART intervention. This allowed us to perform a subgroup analysis of outcomes from IVF treatment only, but we were unable to perform subgroup analyses for other ART methods due to insufficient numbers of studies reporting outcomes for these interventions.

A sensitivity analysis was performed adjusting for age as a potential confounding factor, and then repeated after excluding studies using oocyte donation cycles in order to assess heterogeneity between these and other patients.

Results

Twenty-one studies were included in the meta-analysis (Trapp *et al*., 1986; Harrison *et al*., 1990; Elenbogen *et al*., 1991; Pattinson *et al*., 1991; Agnani *et al*., 1994; Hughes *et al*., 1994; Sharara *et al*., 1994; Maximovich and Beyler, 1995; Gustafson *et al*., 1996; Sterzik *et al*., 1996; Van Voorhis *et al*., 1996; Feichtinger *et al*., 1997; El-Nemr *et al*., 1998; Weigert *et al*., 1999; Crha *et al*., 2001; Winter *et al*., 2002; Crha *et al*., 2003; Tiboni *et al*., 2004; Lintsen *et al*., 2005; Wright *et al*., 2006; Soares *et al*., 2007). Four of these studies reported data on live birth rates per cycle (Pattinson *et al*., 1991; Sharara *et al*., 1994; Gustafson *et al*., 1996; Lintsen *et al*., 2005). Eighteen studies reported data on clinical pregnancy rates per cycle (Trapp *et al*., 1986; Harrison *et al*., 1990; Elenbogen *et al*., 1991; Pattinson *et al*., 1991; Agnani *et al*., 1994; Hughes *et al*., 1994; Sharara *et al*., 1994; Gustafson *et al*., 1996; Sterzik *et al*., 1996; Van Voorhis *et al*., 1996; Feichtinger *et al*., 1997; El-Nemr *et al*., 1998; Weigert *et al*., 1999; Crha *et al*., 2001, 2003; Tiboni *et al*., 2004; Wright *et al*., 2006; Soares *et al*., 2007). Seven studies reported data on miscarriage rates per pregnancy (Harrison *et al*., 1990; Pattinson *et al*., 1991; Hughes *et al*., 1994; Maximovich and Beyler, 1995; Gustafson *et al*., 1996; Winter *et al*., 2002; Soares *et al*., 2007), and three studies examined the risk of ectopic pregnancy (Elenbogen *et al*., 1991; Agnani *et al*., 1994; Gustafson *et al*., 1996).

Fifteen studies reported data on the effect of female cigarette smoking on IVF alone, allowing for subgroup analysis (Trapp *et al*., 1986; Elenbogen *et al*., 1991; Pattinson *et al*., 1991; Agnani *et al*., 1994; Hughes *et al*., 1994; Sharara *et al*., 1994; Maximovich and Beyler, 1995; Gustafson *et al*., 1996; Sterzik *et al*., 1996; Feichtinger *et al*., 1997; El-Nemr *et al*., 1998; Weigert *et al*., 1999; Crha *et al*., 2001, 2003; Lintsen *et al*., 2005). Of the remaining studies, one reported on oocyte donation cycles (Soares *et al*., 2007), two combined data for IVF and ICSI (Neal *et al*., 2005; Wright *et al*., 2006), one combined data for IVF and GIFT (Harrison *et al*., 1990), one reported results for IVF, GIFT and ZIFT (Van Voorhis *et al*., 1996), one reported results after IVF, ICSI and GIFT (Winter *et al*., 2002) and the final study used ICSI and FIVET (Tiboni *et al*., 2004).

Eighteen of the 21 included studies had a primary aim of investigating the effect of cigarette smoking (with or without other lifestyle factors) on outcomes of ART (Trapp *et al*., 1986; Harrison *et al*., 1990; Elenbogen *et al*., 1991; Pattinson *et al*., 1991; Agnani *et al*., 1994; Hughes *et al*., 1994; Sharara *et al*., 1994; Maximovich and Beyler, 1995; Sterzik *et al*., 1996; Van Voorhis *et al*., 1996; Feichtinger *et al*., 1997; El-Nemr *et al*., 1998; Weigert *et al*., 1999; Crha *et al*., 2001; Winter *et al*., 2002; Lintsen *et al*., 2005; Wright *et al*., 2006; Soares *et al*., 2007). The remaining three studies reported the effect of cigarette smoking although this was not the primary aim: one

Table I Characteristics of studies included in the meta analysis

Study	Design	Study population	Method	Treatment protocol	Inclusion criteria	Exclusion criteria	Smoking definition
Agnani *et al.* (2003)	Retrospective	47 non-smokers (62 cycles)	IVF	LHRH agonist + hMG	1. Tubal infertility due to salpingitis	None stated	≥10 cigarettes/day
		24 smokers (38 cycles)					Ascertained by telephone/letter
Crha *et al.* (2001)	Prospective	90 non-smokers	IVF	FSH + hMG + GnRH analogue	Patients undergoing IVF 1997–1999.	None stated	Smokers ≥0–1 cigarettes/day
		40 smokers					Ascertained by questionnaire and urinary cotinine
Crha *et al.* (2003)	Prospective	38 smokers	IVF	HMG + FSH + GnRH analogue	Women undergoing infertility treatment, selected for age and smoking habits	None stated	None stated
		38 non-smokers					Ascertained by questionnaire and urinary cotinine
El-Nemr *et al.* (1998)	Retrospective	108 non-smokers	IVF	GnRH agonist + HCG + hMG or FSH	All women undergoing consecutive IVF-ET cycles June 1995-February 1996	1. ICSI intervention	Any number per day
		65 smokers					Ascertained by interview
Elenbogen *et al.* (1991)	Prospective	21 non-smokers	IVF	GnRH agonist + HMG + FSH + HCG	1. <37 years age	None stated	>15 cigarettes/day
		20 smokers			2. Normal ovulatory cycles		Ascertained by questionnaire
					3. Normal spermiograms in male		
					4. Infertility due to tubal disease		
Feichtinger *et al.* (1997)	Prospective	399 non-smokers	IVF	None stated	IVF patients undergoing first cycle from January 1996	None stated	None stated
		142 smokers					Ascertained by medical history
Gustafson *et al.* (1996)	Prospective	50 non-smokers	IVF	Clomiphene citrate + HMG + HCG	1. At least 1 cleaved and transferred embryo	1. Uterine abnormalities	At least 10 cigarettes/day
		50 smokers			2. At least 1 unfertilized oocyte	2. Endometriosis	Ascertained by medical history
					3. Spontaneous ovulatory cycles	3. PCOS	
					4. Immunity against rubella	4. Treatment with thyroid hormone	
					5. Seronegativity for HIV	5. Treatment with corticosteroid	
					6. Normal male spermiogram		

Harrison *et al.* (1990)	Prospective	542 non-smokers	IVF and GIFT	Clomiphene citrate + HMG + HCG	1. Patients undergoing IVF/GIFT January-June 1988	1. Unstable smoking habit	≥1 cigarette/day. Stable for >1 month pretreatment.
		108 smokers			2. Age <40 years		Ascertained by medical history
Hughes *et al.* (1994)	Prospective	119 non-smokers (182 cycles)	IVF	GnRH agonist + HMG + HCG	1. Patients undergoing IVF March 1990 and April 1992.	1. Alternative forms of stimulation (clomiphene alone, clomiphene and hMG, unmedicated)	>1 cigarette/day
		96 smokers (155 cycles)			2. Stimulated using GnRH analogue flare up regime		Ascertained by questionnaire
Lintsen *et al.* (2005)	Retrospective	4706 non-smokers	IVF	Not stated	1. Infertility>1 year	1. ICSI intervention	>1 cigarette/day for >1 year
		3617 smokers			2. First cycle only	2. Unstimulated cycles	Ascertained by medical records/questionnaire
						3. ZIFT	
						4. GIFT	
						5. Gamete and embryo donation	
						6. Frozen embryo transfers	
Maximovich and Beyler (1995)	Retrospective	210 non-smokers	IVF	GnRH agonist + HMG	1. Luteal phase GnRH-a supression with hMG	None stated	None stated
		43 smokers			2. TUDOR		Ascertained by questionnaire
					3. Cycles resulting in ET after TUDOR		
Pattinson *et al.* (1991)	Retrospective	236 non-smokers	IVF	Clomiphene citrate + HMG + HCG	All couples who had undergone first attempt IVF treatment between March 1984 to March 1989.	None stated	≥1 cigarette/day
		124 smokers					
Sharara *et al.* (1994)	Retrospective	73 non-smokers	IVF	GnRH analogue + HMG + HCG	1. Age 35–39	None stated	Currently smoking cigarettes
		29 smokers			2. Strictly tubal factor infertility		Ascertained by questionnaire
					3. Normal CC challenge tests within 1 year of IVF cycle		
Soares *et al.* (2007)	Retrospective	680 non-smokers	IVF following oocyte donation	GnRH agonist + FSH ± HMG + HCG	All oocyte donation cycles performed between January 2002 and June 2005	1. Heavy smoking oocyte donor	>10 cigarettes/day
		44 smokers				2. Actively smoking male partner	

Continued

Table I *Continued*

Study	Design	Study population	Method	Treatment protocol	Inclusion criteria	Exclusion criteria	Smoking definition
Sterzik *et al.* (1996)	Prospective	68 non-smokers	IVF	HMG + HCG	1. Stricly tubal factor infertility	None stated	Follicular fluid concentration: s > 50 ng/ml, 20 < ps < 50 ng/ml, ns < 20 ng/ml in addition to interview based questionnaires.
		103 smokers			2. Normal spermiogram in male partner 3. Infertility>1 year 4. Positive follicle aspiration after hormonal stimulation		
Tiboni *et al.* (2004)	Prospective	43 non-smokers	ICSI and FIVET	GnRH agonist + FSH + HCG	Patients undergoing ART	1. Taking micronutrient supplementation	≥1 cigarette/day
		17 smokers					Ascertained by questionnaire
Trapp *et al.* (1986)	Prospective	76 non-smokers	IVF	Clomiphene citrate + HMG + HCG	Patients receiving IVF treatment 1984–1985	None stated	None stated
		38 smokers					Ascertained by questionnaire and SCN concentration in serum and follicular fluid
Van Voorhis *et al.* (1996)	Retrospective	351 non-smokers	IVF, GIFT and ZIFT	GnRH agonist + HCG	All first assisted reproduction cycles January 1989 to July 1994	1. Women with cancelled cycles	Smoking during ART cycle.
		37 smokers				2. Donor oocytes	Ascertained by questionnaire
Weigert *et al.* (1999)	Retrospective	634 non-smokers	IVF	Clomiphene citrate + HMG or	All IVF patients	None stated	At least 1 cigarette/day
		200 smokers		GnRH agonist + FSH			Ascertained by questionnaire
Winter *et al.* (2002)	Retrospective	1060 non-smokers	IVF, ICSI and GIFT	HMG + clomiphene citrate or	All pregnancies achieved by embryo transfers 1994–1999	None stated	Not stated
		136 smokers		GnRH agonist + HMG			
Wright *et al.* (2006)	Retrospective	306 non-smokers	IVF and ICSI	GnRH analogue + FSH + HCG	First cycle IVF treatment 31 December 2002 to 6 April 2004.	1. Donor oocytes	Currently smoking
		36 smokers					Ascertained by questionnaire and physicians record

study investigated ascorbic acid supplementation in ART (Crha *et al.*, 2003), one investigated hyperandrogenism (Gustafson *et al.*, 1996) and one investigated concentrations of fat soluble vitamins and micronutrients between smokers and non-smokers (Tiboni *et al.*, 2004).

Of the 28 studies included in the systematic review and meta-analysis, 9 included information regarding male partner smoking status (Pattinson *et al.*, 1991; Hughes *et al.*, 1994; Joesbury *et al.*, 1998; Klonoff-Cohen *et al.*, 2001; Zitzmann *et al.*, 2003; Neal *et al.*, 2005; Soares *et al.*, 2007; Wright *et al.*, 2006; Zenzes *et al.*, 1997).

Fertilization rates were reported in many studies as continuous data with mean percentage oocytes fertilized and standard deviation, omitting the total number of oocytes retrieved and the number of cycles undertaken for each group. We therefore consider it to be statistically naïve to attempt to combine these results between studies, and accordingly a meta-analysis for fertilization rates has not been attempted. Data for this outcome has been included in the systematic review.

Quality of included studies

A study with the highest quality for the purpose of this review would be of prospective design with sample sizes based upon a power calculation. There would be no significant difference in age between smoking and non-smoking groups, and patients above the age of 40 would not be included. Results would be reported for only the first cycle for each patient in order to eliminate the confounding effect of several attempts: continuation of IVF depends on predictors of success observed in the first cycle (Stolwijk *et al.*, 1996). Studies would also report the smoking status of the male partner. Table II displays the quality of the included studies based upon these criteria.

Live birth rate per cycle

Data from four studies were combined in the meta-analysis to determine the pooled OR for live birth rate regardless of method of assisted reproduction. The four studies included in this meta-analysis reported data on 3252 cycles for smokers and 4213 cycles for non-smoking controls. The sample size varied across the trials from 100 to 6903 cycles. Smoking patients demonstrated a significantly decreased live birth rate per cycle (OR 0.54, 95% CI 0.30–0.99).

Clinical pregnancy rate per cycle

The eighteen studies included in this meta-analysis reported data on cycles for 1284 smokers and 3959 matched controls. The sample size varied across the trials from 41 to 834 cycles. The clinical pregnancy rate per cycle was significantly lower for smokers (OR 0.56, 95% CI 0.43–0.73) (Fig. 2).

Spontaneous miscarriage rate per clinical pregnancy

The seven studies included reported data on pregnancies of 211 smokers and 1688 matched controls. The sample size varied across the trials from 23 to 1196. A significant increase in the odds of miscarriage per pregnancy was observed in the group of smokers (OR 2.65, 95% CI 1.33–5.30) (Fig. 3).

Ectopic pregnancy rate per clinical pregnancy

The data from three studies provided no evidence for statistical heterogeneity and therefore a fixed effect model was used. The studies reported data on pregnancies for 10 smokers and 42 non-smoking controls. The ectopic pregnancy rate per clinical pregnancy was significantly higher in smokers (OR 15.69, 95% CI 2.87–85.76), although the large CI is a likely reflection of the small sample size.

Subgroup analysis

Subgroup analysis was carried out for patients undergoing IVF treatment only.

Live birth rate per cycle

The treatment intervention for all studies reporting live birth rates used IVF and therefore no subgroup analysis was required.

Clinical pregnancy rate per cycle

The subgroup analysis included data for 1042 smokers and 2037 non-smokers. The clinical pregnancy rate per cycle remained significantly lower for smokers (OR 0.57, 95% CI 0.42–0.77).

Spontaneous miscarriage rate per cycle

Data on cycles for 52 smokers and 158 non-smoking controls was pooled. There was no longer evidence of a significant difference in odds of miscarriage (OR 2.99, 95% CI 0.94–9.47).

Ectopic pregnancy rate per cycle

All studies reporting ectopic pregnancy rates per clinical pregnancy used conventional IVF so no subgroup analysis was required.

Sensitivity analysis: age

A sensitivity analysis was performed based on the age of patients, and the meta-analysis repeated after excluding all studies where age was likely to be a confounding factor. Three studies were excluded because of a statistically significant difference in age between smokers and non-smokers (Van Voorhis *et al.*, 1996; Weigert *et al.*, 1999; Wright *et al.*, 2006). A further six studies were excluded for failing to state whether any significant difference in age between study groups existed (Trapp *et al.*, 1986; Harrison *et al.*, 1990; Agnani *et al.*, 1994; Feichtinger *et al.*, 1997; Tiboni *et al.*, 2004; Lintsen *et al.*, 2005). Of the remaining studies, a further three studies were excluded for including patients over the age of 40 (Maximovich and Beyler, 1995; Winter *et al.*, 2002; Soares *et al.*, 2007). The results for one study (El-Nemr *et al.*, 1998) were stratified by age and could therefore be adjusted to include only those patients below 40 years of age.

The studies excluded by the sensitivity analysis for age included all those that did not use conventional IVF treatment only. Therefore, no further subgroup analysis was required.

Live birth rate per cycle

Three studies were combined for live birth rate per cycle for 203 smokers and 359 non-smokers. There was no longer any evidence for a difference in live birth rate between smokers and non-smokers (OR 0.41, 95% CI 0.14–1.14).

Table II Quality of studies included in the meta-analysis

Study	Prospective design	Sample size calculation	Age >40 excluded	Significant age difference	Mean age (SD)	1 cycle /patient	First cycle only	Partner smoking status
Agnani *et al.* (1994)	No	No	No	Not stated	Range 25–41	No	No	No
Crha *et al.* (2001)	Yes	No	Yes	No	29.4 (4.3)	Yes	No	No
Crha *et al.* (2003)	Yes	No	Not stated	No	NS: 27.3 (8.5) S: 26.8 (10.0)	Yes	No	No
El-Nemr *et al.* (1998)	No	No	No: result stratified by age	No	NS: 32.5 (4.5) S: 31.6 (4.8)	Yes	No Stratified by cycles	No
Elenbogen *et al.* (1991)	Yes	No	Yes	No	NS: 32.6 (1.6) S: 33.5 (1.8)	Yes	No	No
Feichtinger *et al.* (1997)	Yes	No	Not stated	Not stated	Not stated	Yes	Yes	No
Gustafson *et al.* (1996)	Yes	No	Yes	No	NS: median 34 (range 25–37) S: median 33 (range 25–37)	Yes	No	No
Harrison *et al.* (1990)	Yes	No	Yes	Not stated	Not stated	Yes	No	No
Hughes *et al.* (1994)	Yes	Yes	Not stated	No	NS: 34.3 (2.88) S: 33.5 (3.31)	No	No	Yes
Lintsen *et al.* (2005)	No	No	No	Not stated	32.8 (3.9)	Yes	Yes	No
Maximovich and Beyler (1995)	No	No	Not stated	No	NS: 35.5 (4.4) S: 36.3 (4.5)	Yes	No	No
Pattinson *et al.* (1991)	No	Yes	Not stated	No	NS: 33.1 (3.8) S: 32.6 (2.9)	Yes	Yes	Yes
Sharara *et al.* (1994)	No	No	Yes	No	NS: 37.2 (1.3) S: 37.8 (1.1)	Yes	No	No
Soares *et al.* (2007)	No	No	No	No	NS: 39.7 (5.1) S: 38.9 (4.9)	Yes	Yes	Yes
Sterzik *et al.* (1996)	Yes	No	Yes	No	NS: 32.5 (4.1) S: 32.4 (4.3)	Yes	Yes	No
Tiboni *et al.* (2004)	Yes	No	No	Not stated	NS: 34.6 S: 35.2	Yes	No	No
Trapp *et al.* (1986)	Yes	No	Yes	Not stated	NS: 34.2 (0.5) S: 33.8 (1.4)	Yes	No	No
Van Voorhis *et al.* (1996)	No	No	Not stated	Not stated	NS: 32.9 (4.2) S: 31.5 (3.6)	Yes	Yes	No
Weigert *et al.* (1999)	No	No	Not stated	Yes	NS:34.19 (5.33) S: 33.27 (4.93)	Yes	No	No
Winter *et al.* (2002)	No	No	No	No	32.7 (4.7)	Yes	No	No
Wright *et al.* (2006)	No	Yes	No Stratified by age	Yes	Not stated	Yes	Yes	Yes

SD, standard deviation.

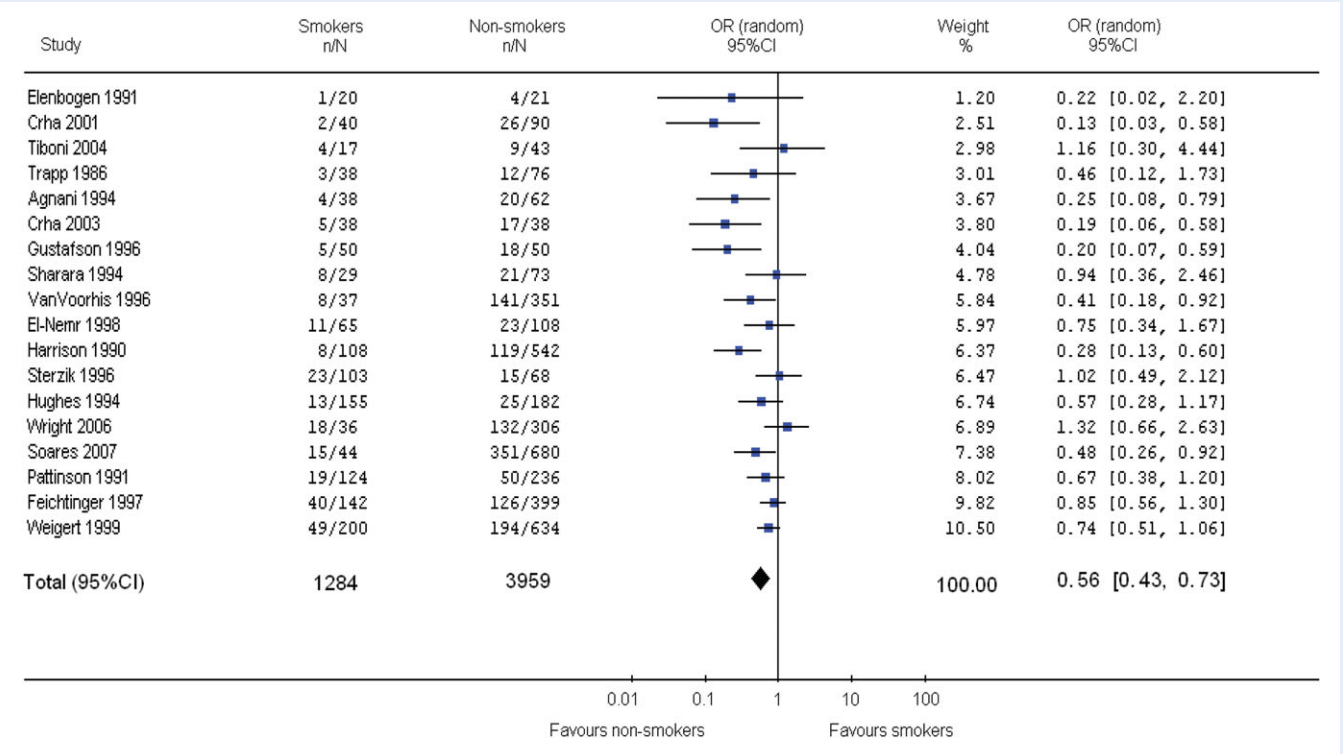

Figure 2 Odds ratio of clinical pregnancy rate per cycle.

Total events: 236 (smokers), 1303 (non-smokers). Test for heterogeneity: $\chi^2 = 33.27$, df = 17 ($P = 0.01$), $I^2 = 48.9\%$. Test for overall effect: $z = 4.26$ ($P < 0.0001$).

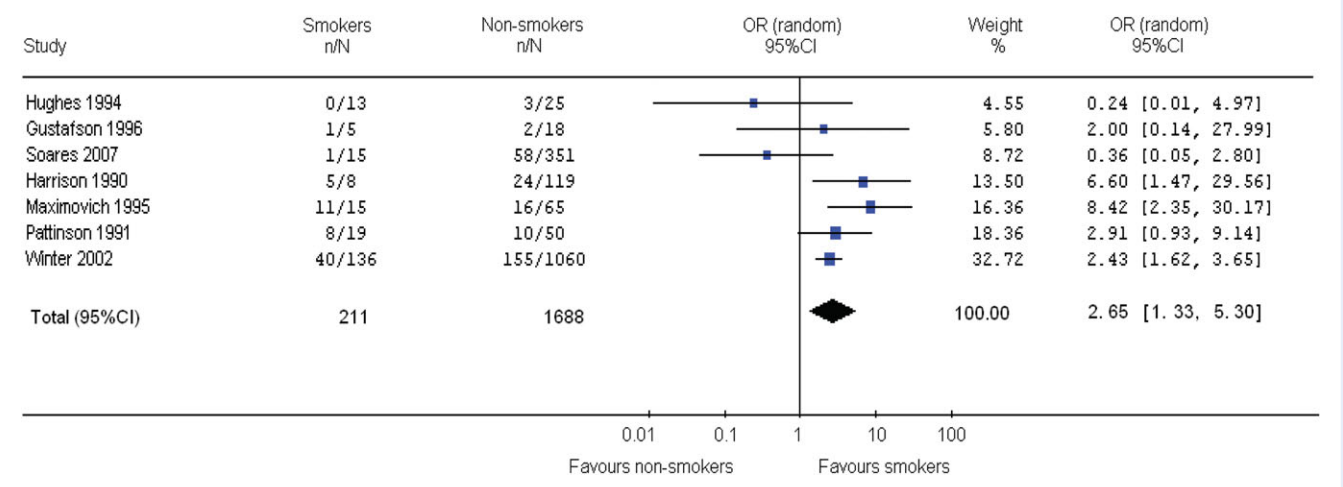

Figure 3 Odds ratio of miscarriage per pregnancy.

Total events: 66 (smokers), 268 (non-smokers). Test for heterogeneity: $\chi^2 = 10.98$, df = 6 ($P = 0.09$), $I^2 = 45.4\%$. Test for overall effect: $z = 2.77$ ($P = 0.006$).

Clinical pregnancy rate per cycle

Data from nine studies was pooled, and reported on the clinical pregnancy rate in 621 cycles of smokers and 859 cycles of non-smokers. The clinical pregnancy rate per cycle remained significantly lower for smokers (OR 0.51, 95% CI 0.32–0.79).

Spontaneous miscarriage rate per clinical pregnancy

Three studies reported on miscarriage rates for pregnancies in a total of 37 smokers and 93 non-smokers. There was no longer any evidence for a difference in miscarriage rate per pregnancy (OR 1.88, 95% CI 0.55–6.37).

Ectopic pregnancy rate per pregnancy

Two studies were available to evaluate the odds of ectopic pregnancy per pregnancy after sensitivity analysis. These reported on pregnancies of six smokers and 22 non-smokers. The ectopic pregnancy rate remained significantly higher for smokers (OR 14.70, 95% CI 1.53–141.15).

Sensitivity analysis had differing effects on the evidence of statistical heterogeneity for each outcome of ART, as evidenced by chi-squared statistic, *P*-value and the I^2 statistic. There was little change in evidence of statistical heterogeneity for live birth rate and clinical pregnancy rate. There was a substantial decrease in statistical heterogeneity for miscarriage rate, and a slight increase for ectopic pregnancy rate.

Sensitivity analysis: oocyte donation cycles

A further sensitivity analysis was conducted to exclude the only study that reported on clinical pregnancy rate and spontaneous miscarriage rate for oocyte donation cycles (Soares *et al*., 2007), in order to assess heterogeneity between these and other patients.

Clinical pregnancy rate per cycle

Sensitivity analysis had a negligible effect upon the odds of achieving clinical pregnancy between smokers and non-smokers (OR 0.57, 95% CI 0.43–0.75). There was very little change in evidence of statistical heterogeneity for this measure.

Spontaneous miscarriage rate per clinical pregnancy

Five studies reported on miscarriage rates per pregnancy of 196 smokers and 1337 non-smokers. The odds of miscarriage increased by 0.4 after sensitivity analysis (OR 3.05, 95% CI 1.74–5.73), and there was a small decrease observed in the measures of statistical heterogeneity ($\chi^2 = 7.17$, df = 5, $I^2 = 30.3\%$).

Fertilization rate

Data for fertilization rate has been included in the systematic review and can be seen in Table III. Of the 17 studies that reported fertilization rates per cycle ART, the majority failed to find any significant difference in fertilization rates between smoking and non-smoking groups (Trapp *et al*., 1986; Pattinson *et al*., 1991; Hughes *et al*., 1994; Sharara *et al*., 1994; Sterzik *et al*., 1996; Van Voorhis *et al*., 1996; El-Nemr *et al*., 1998; Joesbury *et al*., 1998; Weigert *et al*., 1999; Neal *et al*., 2005; Wright *et al*., 2006). A significant decrease in fertilization rates for smokers was observed in four studies (Elenbogen *et al*., 1991; Rosevear *et al*., 1992; Crha *et al*., 2001; Tiboni *et al*., 2004), while one study found a significantly higher fertilization rate for smokers (Crha *et al*., 2003). One study found a significantly higher fertilization rate only for smokers over the age of 35 (Zenzes *et al*., 1997).

Male partner smoking status

Male partner smoking status is another factor responsible for clinical heterogeneity between studies; however, insufficient data was reported to allow for sensitivity or subgroup analysis. Only nine of

Table III Fertilization rates per cycle

Study	Sample size	Mean % oocytes fertilized: smokers (SD)	Mean % oocytes fertilized: non-smokers (SD)
Crha *et al*. (2001)	40 smokers 90 non-smokers	47.8 (40.3)	68.2 (33.2)
Crha *et al*. (2003)	38 smokers 38 non-smokers	86.0	70.3
El-Nemr *et al*. (1998) (first cycle only)	33 smokers 68 non-smokers	45.4	45.1
Elenbogen *et al*. (1991)	20 smokers 21 non-smokers	40.9	61.7
Hughes *et al*. (1994)	155 smokers 182 non-smokers	65.7 (37.0)	64.3 (36.3)
Joesbury *et al*. (1998)	74 smokers 391 non-smokers	60 (20)	61 (20)
Neal *et al*. (2005)	39 smokers 146 non-smokers	57	63
Pattinson *et al*. (1991)	124 smokers 236 non-smokers	65.9	68.5
Rosevear *et al*. (1992)	32 non-smokers 13 smokers	44	72
Sharara *et al*. (1994)	29 smokers 73 non-smokers	78 (11)	82 (14)
Sterzik *et al*. (1996)	103 smokers 68 non-smokers	67.9	67.6
Tiboni *et al*. (2004)	17 smokers 43 non-smokers	55.9	71.5
Trapp *et al*. (1986)	36 smokers 66 non-smokers	45.1 (6.6)	49.9 (4.7)
Van Voorhis *et al*. (1996)	37 smokers 351 non-smokers	49.9 (25.4)	54.1 (25.0)
Weigert *et al*. (1999)	200 smokers 634 non-smokers	60.8	60.9
Wright *et al*. (2006)	36 smokers 316 non-smokers	64.6 (21.0)	64.9 (23.5)
Zenzes *et al*. (1997)	130 non-smokers 74 smokers	Age <35: 67.9 Age >35: 78.1	Age <35: 69.4 Age >35: 67.5

the 28 studies included in the systematic review and meta-analysis contained any information regarding male partner smoking status (Pattinson *et al*., 1991; Hughes *et al*., 1994; Zenzes *et al*., 1997; Joesbury *et al*., 1998; Klonoff-Cohen *et al*., 2001; Zitzmann *et al*., 2003; Neal *et al*., 2005; Wright *et al*., 2006; Soares *et al*., 2007). Of these, one reported a non-significant effect upon live birth rate (Pattinson *et al*., 1991). Five studies reported the effect of male smoking upon clinical pregnancy rate (Pattinson *et al*., 1991; Joesbury *et al*., 1998; Klonoff-Cohen *et al*., 2001; Zitzmann *et al*., 2003; Neal *et al*., 2005), and of these three found evidence of a significant difference in pregnancy rate between male smokers and non-smokers (Joesbury *et al*., 1998; Zitzmann *et al*., 2003; Neal *et al*., 2005). Of the three studies that reported fertilization rates between male partner smokers and non-smokers (Hughes *et al*., 1994; Joesbury *et al*., 1998; Neal *et al*., 2005), none found any evidence of a significant effect. Of the remaining three studies, one required all participating male partners to be non-smokers to allow examination of female smoking effect only (Soares *et al*., 2007), and the remaining two studies stated the smoking status of the males but undertook no analysis of the effect of male smoking, reporting insufficient data to allow this to be done (Zenzes *et al*., 1997; Wright *et al*., 2006).

A funnel plot was generated to detect any publication bias. This was generated for the outcome of clinical pregnancy rate only as there were insufficient numbers of studies reporting on other outcome measures. The funnel plot generated suggests some presence of publication bias: although there are equal numbers of studies either side of the vertical line, the plot is not symmetrical (Fig. 4). While publication bias is one explanation for this asymmetry it is important to also consider other sources of selection bias, heterogeneity of studies, methodological limitations of studies and chance (Higgins and Green, 2005).

Discussion

The results of this meta-analysis provide evidence of a significant negative effect of cigarette smoking by women at the time of infertility treatment, upon clinical outcomes of ART.

Compared with non-smokers, cigarette smokers demonstrated a significantly lower live birth rate per ART cycle (OR 0.54, 95% CI 0.30–0.99). Surprisingly, only four studies reported results for this outcome. Future studies should endeavour to report live birth rates because the goal of ART from the perspective of the patient is to overcome infertility by achieving live birth. While it is important for physicians to be aware of proximate treatment-orientated success rates that are often reported, such as oocyte retrieval or fertilization rates, it is also important that more distal patient-orientated outcomes such as live birth rates are reported. Without this information, prospective patients cannot make informed decisions about lifestyle factors that could affect their ART success.

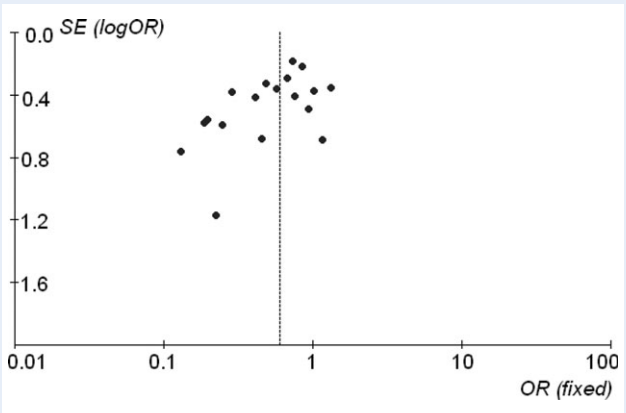

Figure 4 Funnel plot of the studies used to determine the odds ratio of clinical pregnancy rate per cycle ART.The vertical line represents the pooled estimate of the OR 0.56.

Results from one study included in the meta-analysis for live birth rate (Lintsen *et al*., 2005) are particularly significant, and the high quality design of this study adds significant weight to the evidence. The authors examined the success rate of IVF in 8457 women from all IVF clinics in the Netherlands, included patients undergoing first IVF cycle only, and classified patients as smokers only if they had a stable smoking habit for at least 1 year prior to treatment. In this study, the odds of live birth for smokers was 0.73 that of non-smokers. This study shows the narrowest CI (0.64–0.84) and was given the highest weight (43.14) in the meta-analysis.

Although not included in the meta-analysis for not reporting raw data, one other study investigated the effect of cigarette smoking on live birth rate and found the risk of smokers not achieving a live birth to be significantly higher than non-smokers [Relative risk (RR) 2.51, 95% CI 1.11–5.67] (Klonoff-Cohen *et al*., 2001).

In order to examine the effect of cigarette smoking on ART it is also important to report clinical pregnancy rate. Although a treatment-orientated rather than patient-orientated outcome, clinical pregnancy rate is not confounded by the complications of smoking observed in the second and third trimesters of both spontaneous and ART pregnancies, that can affect live birth rate. Therefore, measuring the clinical pregnancy rate allows an objective evaluation of the efficacy of the ART cycle itself. The meta-analysis detected a significantly lower clinical pregnancy rate per cycle ART cycle among smokers (OR 0.56, 95% CI 0.43–0.73). It is interesting to note that this result is very similar to that reported in previous meta-analyses (Feichtinger *et al*., 1997; Augood *et al*., 1998). Augood *et al*. also reported a significantly lower clinical pregnancy rate amongst smokers (OR 0.66, 95% CI 0.49–0.88), and Feichtinger *et al*. found that smokers were required to undergo 1.79 (95% CI 1.24–2.59) IVF–embryo transfer cycles compared with non-smokers in order to achieve pregnancy.

Four additional studies investigated clinical pregnancy rates, but could not be included in the meta-analysis: one study did not report raw data, but found the risk of a smoker never achieving a pregnancy compared with a non-smoker to be significantly increased (RR 2.71, 95% CI 1.37–5.35) (Klonoff-Cohen *et al*., 2001). Three other studies reported clinical pregnancy rates per embryo transfer rather than per cycle ART. One found a significant difference in pregnancy rate [20% in 39 smokers compared with 48.3% in 146 non-smokers (Neal *et al*., 2005)], and two found no significant difference between smoking and non-smoking groups (Maximovich and Beyler, 1995; Motejlek *et al*., 2006).

Of patients that achieved a pregnancy following ART, smokers had significantly higher odds of spontaneous miscarriage (OR 2.65, 95% CI 1.33–5.30), and the meta-analysis also found an increased risk of ectopic pregnancy among smokers (OR 15.69, 95% CI 2.87–85.76).

While statistically significant, the evidence for the higher OR observed for ectopic pregnancy rate is less reliable, as evidenced by the very large CI. Since there is no evidence for statistical heterogeneity for this outcome, this is likely to be due to the small sample size as a result of the paucity of studies reporting ectopic pregnancy rates.

The cyclic nature of ART gives rise to many difficulties in defining the exact effect of smoking upon outcomes of ART: the success rate of any outcome is not independent of other outcomes and is a reflection of previous stages in that cycle. Furthermore, the exact mechanisms by which cigarette smoking may exert a detrimental effect upon outcomes of ART are currently relatively unknown. A strong body of evidence indicates that the negative effect of cigarette smoking on fertility is evident in every system involved in the reproductive process (Soares and Melo, 2008). Research to date suggests that there are likely to be several mechanisms taking place at different stages of the assisted reproduction cycle that underlie these effects, with effects on the ovaries, the oocyte and the uterus. Current research suggests that in addition to a reduced ovarian reserve observed in smokers (Sharara *et al*., 1994), cigarette smoking may also impair folliculogenesis by causing an imbalance between pre- and anti-oxidants resulting in oxidative stress in the Graafian follicle (Paszkowski *et al*., 2002). Damage to the oocyte itself is likely to occur as a result of mutagens such as cadmium, cotinine and polycyclic aromatic hydrocarbons that are present in cigarette smoke and have been found in the follicular fluid of female cigarette smokers. Adducts of these compounds have the potential to damage DNA, alter the meiotic spindle of oocytes and affect the function and viability of oocyte granulosa cell complexes (Zenzes *et al*., 1998; Zenzes, 2000). Cigarette smoking has also been shown to decrease the number of mature oocytes retrieved following ovulation induction in ART (Zenzes *et al*., 1997): this may be caused by an increased concentration of vascular endothelial growth factor antagonists impairing angiogenesis of oocytes (Motejlek *et al*., 2006). The decreased fertilization and pregnancy rates often observed in these studies may be due to an increase in the zona pellucida thickness of oocytes observed in cigarette smokers (Shiloh *et al*., 2004), in addition to a reduced uterine receptiveness (Soares *et al*., 2007). Previous studies have also identified antioestrogenic effects of cigarette smoking that may account for increased miscarriage rates: inhibition of granulosa cell aromatase activity in smokers can result in corpus luteal deficiency (Shiverick and Salafia, 1999). Finally, studies in animal models have found tobacco to alter the function of Fallopian tubes therefore increasing the risk of ectopic pregnancy (Saraiya *et al*., 1998).

It is important to acknowledge the limitations of meta-analyses that pool data from observational studies rather than randomized controlled trials. There is more potential for results to be confounded, and it is difficult to ensure that retrospective studies are a true representation of the population. Unfortunately, due to the nature of the research question there is no alternative, and results from these meta-analyses still make a valid contribution to current knowledge and understanding of these issues.

All studies included in this meta-analysis share the common design of comparing a group of patients who smoke cigarettes with a control group that do not. Using this information it is possible to report a general effect of smoking on the outcomes of ART. Two forms of heterogeneity (statistical and clinical) exist between studies and must be accounted for. In this meta-analysis, statistical heterogeneity between studies was compensated for using a random effects model where appropriate.

One of the main factors responsible for clinical heterogeneity was the inclusion of significantly different age groups of smoking and non-smoking women in some studies. Age is one of the most important confounding factors in ART success and has been shown to be a strong predictor of this in numerous studies, with both pregnancy and live birth rates declining with increasing age of the patient (Winston and Handyside, 2007). In addition to the negative effect of age on the ovary, a negative effect of age has also been observed upon uterine receptiveness: ART outcomes were found to be considerably worse in older recipients of oocyte donation cycles (Soares *et al*., 2005). In an attempt to account for this heterogeneity, a sensitivity analysis was performed to exclude all studies that either reported a significant difference in age between groups, failed to state whether a significant age difference existed, or included patients over the age of 40. Surprisingly, when this sensitivity analysis was performed for clinical pregnancy rate, there was little effect on the statistical evidence of heterogeneity for all outcomes. This may be due to the sensitivity analysis significantly decreasing the sample sizes for outcomes, therefore reducing the reliability and power of the chi-squared test to detect heterogeneity. In order to eliminate this confounding factor in future studies, researchers should endeavour to compare outcomes between groups without statistically significant differences in age, and to either exclude older patients from their analyses or to stratify results by age thus allowing separate analyses to be conducted.

Male partner smoking status was identified to be a likely cause of clinical heterogeneity between studies. The vast majority of studies did not report male smoking status, and it was therefore not possible to undertake a sensitivity analysis for this confounding variable. The systematic review revealed conflicting evidence on the effect of male smoking upon clinical pregnancy rates, however, no evidence was found of a significant effect upon either live birth rates or fertilization rates. It is difficult to define the exact effect of male partner smoking status upon outcomes of ART, as the negative sequelae are likely to be as a result of both the direct effect upon spermatozoa and the indirect effect of passive smoking upon the female partner. Research suggests that both factors are significant influences, and that male partner smoking damages DNA of spermatozoa thereby hindering future embryonic development (Rubes *et al*., 1998; Zenzes *et al*., 1999), as well as the detrimental effect to female fertility of passive smoking from an actively smoking male partner (Neal *et al*., 2005).

The definition of smoking varied widely between studies, and this variation is another likely cause of heterogeneity. Some studies defined smokers as those smoking at least fifteen cigarettes per day (Elenbogen *et al*., 1991), or required a stable habit for a year before treatment (Lintsen *et al*., 2005). Other authors defined smokers to be those smoking 0-1 cigarettes per day with no minimum duration of smoking habit (Crha *et al*., 2001). Definitions of smoking in other studies varied between these extremes. It is therefore not possible to comment on outcomes of ART based on degree of smoking habit (number smoked per day), but rather to report results for only current cigarette smoking versus non-cigarette smoking at the time of treatment.

One of the studies included in the meta-analysis reported outcomes in smoking and non-smoking patients for oocyte donation cycles

(Soares *et al*., 2007). The inclusion of this study is questionable due to the uncertainty of the site of the negative effect of smoking, whether it be ovarian, uterine or both. To assess whether this was a source of heterogeneity, a sensitivity analysis was conducted in which this study was excluded. The results showed a negligible effect upon clinical pregnancy rate and a small increase in the odds of miscarriage, indicating that the inclusion of this study did not have a significant effect upon the results.

Other potential confounding factors were rarely reported in studies and did not therefore allow for sensitivity analysis. One such example is BMI: only three of the 21 studies included in this meta-analysis reported BMI for both smoking and non-smoking patients (Gustafson *et al*., 1996; Wright *et al*., 2006; Soares *et al*., 2007), and it was therefore not possible to carry out a sensitivity analysis for this factor. Future studies should report this information along with details of any other potential confounding factors in order to identify and account for significant differences between groups.

Despite the heterogeneity between studies and causes of bias mentioned above, the large sample size gained in this meta-analysis by pooling results between studies compensates to a large extent for this variation.

We performed a subgroup analysis for method of ART in an attempt to further account for clinical heterogeneity between studies. This allowed us to compare outcomes between studies using conventional IVF treatment only, but there were insufficient studies to pool results for any other subgroup of method of ART. This analysis resulted in decreased statistical evidence for heterogeneity, thus confirming that treatment intervention type may be a factor in the heterogeneity of the initially analysed studies.

Subgroup analysis did not result in exclusion of any of the studies originally pooled for live birth rate and ectopic pregnancy rate. After subgroup analysis for IVF treatment only, statistically significant results were observed for clinical pregnancy rate (OR 0.57, 95% CI 0.42–0.77), although the results for spontaneous miscarriage rate were no longer significant (OR 2.99, 95% CI 0.94–9.47). This is likely to be due to excluding the two studies with the largest sample sizes, therefore significantly decreasing the power of meta-analysis.

In conclusion, the current meta-analysis provides evidence for a significantly negative effect of cigarette smoking on clinical outcomes of ART. There is particularly overwhelming evidence for a decreased clinical pregnancy rate amongst smokers, in addition to the strong implication of a negative effect on live birth rate, miscarriage rate, ectopic pregnancy rate and fertilization rate. In order to improve success rates for ART this evidence should be presented to actively smoking women seeking treatment for infertility, along with strong advice to cease smoking.

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