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Secondhand tobacco smoke exposure is associated with increased risk of failed implantation and reduced IVF success

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BACKGROUND: Infertility and early pregnancy loss are prevalent as is exposure to secondhand tobacco smoke (STS). Previous research has suggested a relationship between STS exposure and early pregnancy loss, but studies have been limited by small study sizes and/or imprecise methods for exposure estimation. IVF allows for the collection of follicular fluid (FF), the fluid surrounding the pre-ovulatory oocyte, which may be a more biologically relevant sample media than urine or serum in studies of early reproduction.

METHODS: In a retrospective analysis of a prospective cohort study, we measured cotinine in FF collected during 3270 IVF treatment cycles from 1909 non-smoking women between 1994 and 2003 to examine the relationship between STS exposure and implantation failure.

RESULTS: In adjusted models, we found a significant increase in the risk of implantation failure among women exposed to STS compared with those unexposed [odds ratio (OR) = 1.52; 95% confidence interval (CI) = 1.20–1.92; risk ratio (RR) = 1.17; 95% CI = 1.10–1.25]. We also found a significant decrease in the odds for a live birth among STS-exposed women (OR = 0.75; 95% CI = 0.57–0.99; RR = 0.81; 95% CI = 0.66–0.99).

CONCLUSIONS: Female STS exposure, estimated through the measurement of cotinine in FF, is associated with an increased risk of implantation failure and reduced odds of a live birth.

Key words: environmental tobacco smoke / cotinine / assisted reproduction / *in vitro* fertilization / follicular fluid

Introduction

Infertility and early pregnancy loss (e.g. spontaneous abortion) are prevalent in the USA and worldwide (Norwitz *et al.*, 2001; Chandra *et al.*, 2005), and there is growing concern about adverse reproductive health effects resulting from secondhand tobacco smoke (STS) exposure. STS is a mixture of over 4000 chemicals, more than 60 of which are known or suspected carcinogens or reproductive toxicants (e.g. carbon monoxide, cadmium, lead, benzene, nicotine, radioactive polonium-210; Lindbohm *et al.*, 2002).

Exposure remains widespread. According to a recent report, during 2007–2008, ~88 million non-smokers (NS) in the USA aged 3 and older were exposed to STS based on an objective exposure measure (Centers for Disease Control and Prevention, 2010). An older study estimated that 87.9% of non-tobacco users in the USA were exposed to STS (Pirkle *et al.*, 1996). Yet, the same study reported that only 33% of all women in the USA reported that they were exposed to STS, indicating that many are unaware of their exposure. There is also a lack of public knowledge regarding many of the consequences of STS exposure (Parker and Sharif, 2006). Thus, even minor associations between exposure and fertility or pregnancy outcomes may have a significant impact on public health.

STS exposure is most commonly estimated through self-report or the measurement of cotinine, the primary proximate metabolite of

nicotine, in biological samples. Cotinine is widely accepted as a biomarker of tobacco smoke exposure because of its specificity and relatively long half-life in body fluids (~16 h) compared with nicotine (~2 h; Benowitz et al., 2009). The utility of other biomarkers has been explored (Zenzes et al., 1995). Most of these, however, are not tobacco-specific (e.g. carbon monoxide, cadmium).

In a previous study, self-reported female STS exposure was associated with decreased implantation and pregnancy rates among 225 women undergoing IVF or ICSI (Neal et al., 2005), though this method of exposure assessment may lead to exposure misclassification and biased risk estimates. We recently conducted a study using creatinine-adjusted urinary cotinine to estimate female STS exposure in 921 women undergoing IVF but found no association between exposure and failed fertilization, failed implantation or spontaneous abortion (Meeker et al., 2007a). A case–control study in Sweden did, however, find increased odds of spontaneous abortion in STS exposed women versus unexposed women based on plasma cotinine levels (George et al., 2006).

The increasing use of assisted reproductive technologies, particularly IVF, has improved our ability to study contributors to infertility and early pregnancy loss by allowing the observation of early and discrete stages in the reproduction process. Follicular fluid (FF), the fluid surrounding the pre-ovulatory oocyte, is routinely collected during IVF treatment but is seldom used despite its superior biological relevance as a matrix within which to measure markers of exposure to STS or other environmental agents. Cotinine levels in FF reflect a developing oocyte's direct exposure to constituents of tobacco smoke (i.e. it is a measure of dose at the target tissue). Since the ovarian follicle has no direct blood supply, in order for cotinine and other chemicals to enter FF, they must diffuse through interstitial fluid and/or be transported through thecal and granulosa cells which surround the antrum and oocyte (Fabro, 1978). Gap junctions (non-specific pores between cells) can transport molecules up to 1000 Da in molecular mass, and since cotinine has a molecular mass of only 176.2 Da, these pores are likely involved in the passive transport of cotinine into FF (Weber et al., 2004).

To our knowledge, the only study to rely on FF cotinine to assess the relationship between STS exposure and early reproduction found no significant difference in fertilization or pregnancy rates between active, passive and NS in a small cohort of IVF patients ($n = 197$, 26 of whom were categorized as being exposed to STS; Sterzik et al., 1996). Overall, studies of the effects of STS on fertility and early pregnancy have had differing, but suggestive, results underscoring the need for additional research. The present study was designed to examine the relationship between female STS exposure and failed implantation using cotinine measured in FF as a biomarker of exposure among a large cohort of women undergoing IVF.

Materials and Methods

Study population

Participants in the present study were couples undergoing IVF treatment between August 1994 and June 2003 at one of three Boston-area clinics. Elements of the original study have been described previously (Meeker et al., 2007a,b). Protocols were approved by the Human Research Committees at all participating institutions. Approximately 65% of couples approached agreed to participate in the study. Couples excluded from the study were those who underwent gamete intrafallopian transfer (GIFT) or were gestational carriers, as well as those who required donor oocytes or donor semen. Couples in which the woman self-reported active smoking were also excluded. In addition, treatment cycles where frozen embryos were transferred and those that failed or were discontinued prior to embryo transfer were excluded from the present analysis. After these exclusions, there were 1909 couples, with a total of 3270 treatment cycles, enrolled in the present study. Participants underwent one to six treatment cycles. A self-administered questionnaire was used to obtain information from each couple on medical history and lifestyle factors such as: demographics, medical and reproductive history, smoking history, duration of infertility and STS exposure status.

Treatment outcomes

All IVF treatment and outcome variables were abstracted from the clinic record. When at least one embryo was transferred but human chorionic gonadotrophin (hCG) levels never reached 5.0 mIU/ml, the cycle outcome was defined as a failed implantation. A biochemical pregnancy was defined by a measurement of luteal hCG of 5.0 mIU/ml or greater with no further evidence (e.g. gestational sac, fetal heartbeat) of a continued pregnancy. Clinical pregnancy was determined by ultrasound visualization of a gestational sac or a fetal heartbeat. Outcomes among clinically recognized pregnancies included an ectopic pregnancy (gestation outside of the uterus), a molar pregnancy (placental formation with no fetus), a spontaneous abortion (fetal demise before 20 weeks of gestation), stillbirth (fetal demise at or beyond 20 weeks gestation) or live birth of at least one infant.

Exposure assessment

Physicians and technicians were asked to retain the FF from study participants during egg retrieval for each cycle. FF was aspirated from follicles using a 16 G needle and constant suction from a Rocket pump apparatus. Fluid was collected from the largest visible follicle before using any flushing medium and then transferred to a sterile Petri dish. Oocytes were scanned for and removed. The fluid, normally discarded at this point, was placed into a 15 ml conical tube and centrifuged for 15 min. The supernatant was placed into a clean storage tube, labeled, refrigerated and transferred to the Brigham and Women's Hospital laboratory within 12 h. At the laboratory, the specimens were aliquoted into 2 ml specimens and frozen at −80°C. FF was analyzed for cotinine using a quantitative enzyme-linked immunosorbent assay (ELISA; BioQuant, Inc., San Diego, CA). This single-step, competitive test uses spectrometric measurement to determine cotinine in body fluids. It has a lower reporting limit of 0.3 ng/ml and inter- and intra-assay variations of 4 and 6%, respectively.

Statistical analysis

Data analysis was performed using SAS software (version 9.2; SAS Institute Inc., Cary, NC, USA). Quantified cotinine concentrations below the limit of detection (LOD) were kept as the reported value. Unquantified cotinine concentrations were assigned a value of one-half of the LOD. Although self-reported smokers were omitted from the study, those who may have misreported their smoking status were identified and excluded based on concentrations of cotinine in FF. Treatment cycles were considered to be from an active smoker if the cycle yielded a FF cotinine concentration of ≥10 ng/ml ($n = 81$ cycles), following Fuentes et al. (2010). In a previous study, we established a FF cotinine cut-off point of 1.11 ng/ml to distinguish STS-exposed NS from unexposed NS (Benedict et al., 2011). Thus, in the present study, cycles from STS-exposed NS were defined as those that yielded FF cotinine concentrations of <10 and >1.11 ng/ml ($n = 386$

cycles). Treatment cycles from unexposed NS were defined by FF cotinine concentrations ≤1.11 ng/ml ($n = 2803$ cycles).

After excluding the treatment cycles of active smokers based on FF cotinine concentration, preliminary exploratory analyses were performed to evaluate variable distributions and to assess bivariate relationships among key covariates. Variables considered as potential confounders were female age, BMI, ethnicity, primary infertility diagnosis, site of treatment, year of treatment, months spent trying to get pregnant, whether the woman had experienced a previous live birth, ampules of gonadotrophins, down-regulation protocol, use of ICSI, use of assisted hatching, number of embryos transferred, day of embryo transfer, number of oocytes retrieved and alcohol consumption. Bivariate relationships between each covariate and the exposure and outcome variables were examined to identify covariates to include in the multivariate models. Covariates included in the final models were considered to be biologically or clinically important in models in which they were not statistically significant (Hosmer and Lemeshow, 1989). The same covariates were included in each model to maintain consistency.

The relationship between STS exposure and implantation failure was initially modeled using only data from subjects' first treatment cycles (to maintain consistency with previous studies that have been conducted on this topic), followed by analysis of data from all cycles to further improve statistical power. Conditional analyses were performed in that only the subset of subjects that had not experienced a failure up to the point of embryo transfer were included. When considering only subjects' first treatment cycles, conditional logistic regression was used to model the association between STS exposure and implantation failure. Generalized estimating equations (GEEs) were used when considering all treatment cycles. GEEs in their simplest form are an extension of logistic regression and are a method of analyzing correlated data (e.g. longitudinal data) that otherwise could be modeled as a generalized linear model (Liang and Zeger, 1986).

As a potentially more clinically relevant measure of effect, we also calculated the odds of a live birth in relation to STS exposure for both first cycle-only data and when considering all the data. When analyzing the multi-cycle data for live birth outcomes, we first used discrete survival analysis, which was carried out by using a logistic regression model and adjusting for cycle number (Cox and Oakes, 1984). Discrete survival analysis censors on the outcome (i.e. a woman can only have the event once), and thus was not used to analyze implantation failure since a woman may experience multiple implantation failures across cycles. We also modeled the multi-cycle live birth data using GEE for comparison.

Since odds ratios (ORs) for common outcomes (≥10%) tend to overestimate the relative risk (McNutt *et al.*, 2003), ORs and risk ratios (RRs) were calculated and compared for both implantation failure and successful live birth outcomes. Thus, both log-binomial and logistic regression models were used to compute effect estimates. Like logistic regression, the log-binomial model is used for the analysis of a dichotomous outcome and models the probability of that outcome (McNutt *et al.*, 2003). Both modeling approaches also assume that the error terms have a binomial distribution. These two approaches differ in that logistic regression uses the logit function as the link between the independent variables and the probability of the outcome. Using the logit function yields an OR. In the log-binomial model, the log link is used, yielding a RR.

Results

Demographic data for the women in this study are presented in Table I. Participants had a mean (SD) age of 35.3 (4.3) years and were predominantly white (90%). Most reported that they had never actively smoked (69%). Male factor and tubal inflammation/occlusion were the most common causes of infertility, accounting for 33 and 20% of primary infertility diagnoses, respectively. The cause of infertility remained unexplained for 18% of couples. Table II presents the treatment outcomes for couples in the study. Just over one-half (53%) of couples experienced a failed implantation in their first IVF cycle and 32% of initial treatment cycles resulted in a live birth.

The relationship between STS exposure and implantation failure is presented in Table III. In crude and adjusted models, we observed a significant increase in the risk of failed implantation among women exposed to STS versus those unexposed when considering only each subject's first treatment cycle as well as when considering all cycles. We also observed a relationship between STS exposure and IVF treatment success (i.e. live birth; Table IV). In adjusted models, STS exposure was associated with a suggestive decline in the odds of a live birth when considering only each subject's first treatment cycle. When considering all cycles, there was a statistically significant reduction in the odds of a successful IVF cycle in relation to STS exposure.

When comparing ORs and RRs in our results, ORs yielded stronger effected estimates than RRs in all analyses performed (i.e. ORs were

Table I Study demographics for 1909 self-reported non-smoking women undergoing IVF who proceeded to embryo transfer.

Female age at first cycle, mean (SD)	35.3 (4.3)
Race, *n* (%)[a]	
White	1716 (89.9)
Non-white	191 (10.0)
Smoking status (self-report), *n* (%)	
Never smoker	1319 (69.1)
Ex-smoker	590 (30.9)
Primary infertility diagnosis, *n* (%)[b]	
Male factor	637 (33.4)
Ovulatory	230 (12.0)
Endometriosis	245 (12.8)
Tubal inflammation/occlusion	386 (20.2)
Cervical/uterine	66 (3.5)
Unexplained	342 (17.9)
Year of first cycle treatment, *n* (%)	
1994	5 (0.3)
1995	319 (16.7)
1996	340 (17.8)
1997	212 (11.1)
1998	6 (0.3)
1999	178 (9.3)
2000	195 (10.2)
2001	284 (14.9)
2002	267 (14.0)
2003	103 (5.4)

[a]Information on race was missing for two subjects.
[b]Information on primary infertility diagnosis was missing for three subjects.

Table II Outcome of IVF treatment cycles for 1909 self-reported non-smoking women who proceeded to embryo transfer.

Reason for failure	First cycles, *n* (%)	All cycles, *n* (%)
Failure of implantation (i.e. never achieved biochemical pregnancy)	1013 (53.1)	1812 (55.4)
Failure of development		
Biochemical pregnancy but never achieved clinical pregnancy	153 (8.0)	271 (8.3)
Clinical pregnancy was molar	1 (0.1)	1 (0.0)
Clinical pregnancy was ectopic	24 (1.3)	43 (1.3)
Clinical pregnancy was therapeutically aborted	2 (0.1)	3 (0.1)
Clinical pregnancy was spontaneously aborted	104 (5.4)	197 (6.0)
Fetus was stillborn	6 (0.3)	10 (0.3)
Successful live birth	606 (31.7)	933 (28.5)
Total	1909 (100)	3270 (100)

Table III ORs and RRs with 95% CIs for implantation failure associated with female STS exposure based on cotinine concentrations in FF.

	Model	
	Crude	Adjusted[a]
First cycle only[b]		
OR, 95% CI (*P*-value)	2.23, 1.66–3.00 (<0.0001)	1.59, 1.17–2.17 (0.004)
RR	1.37, 1.25–1.51 (<0.0001)	1.17, 1.07–1.28 (0.0005)
All cycles[c]		
OR	1.93, 1.54–2.42 (<0.0001)	1.52, 1.20–1.92 (0.0005)
RR	1.31, 1.21–1.41 (<0.0001)	1.17, 1.10–1.25 (<0.0001)

[a]Adjusted for age, BMI, year of treatment and down-regulation protocol.
[b]Female STS exposure was present during 224 initial treatment cycles.
[c]Female STS exposure was present during 386 total treatment cycles.

always further from the null). Effect estimates were not sensitive to the cotinine cut-point chosen to define the STS-exposed group. For example, similar results were obtained when using the median FF cotinine concentration to define STS exposed or unexposed non-smoking women (not shown).

Discussion

The primary aim of the present study was to determine the association between female STS exposure and implantation failure among couples undergoing IVF. In models adjusted for potential confounders, we found an increased risk of failed implantation among women exposed to STS versus those who were unexposed based on cotinine concentrations measured in FF. As a secondary aim, we also examined the relationship between STS exposure and the odds of a successful live birth as a potentially more clinically relevant effect measure. In our adjusted analysis, women exposed to STS were less likely to have a successful live birth compared with those who were unexposed.

Table IV ORs and RRs with 95% CIs for successful live births associated with female STS exposure based on cotinine concentrations in FF.

	Model	
	Crude	Adjusted[a]
First cycle only[b]		
OR[c], 95% CI (*P*-value)	0.48, 0.35–0.68 (<0.0001)	0.71, 0.50–1.02 (0.06)
All cycles[d]		
OR[e]	0.57, 0.44–0.74 (<0.0001)	0.76, 0.58–0.99 (0.045)
All cycles[d]		
OR[f]	0.57, 0.44–0.73 (<0.0001)	0.75, 0.57–0.99 (0.04)
RR[f]	0.66, 0.54–0.80 (<0.0001)	0.81, 0.66–0.99 (0.04)

[a]Adjusted for age, BMI, year of treatment and down-regulation protocol.
[b]Female STS exposure was present during 224 initial treatment cycles.
[c]Calculated using logistic regression.
[d]Female STS exposure was present during 386 total treatment cycles.
[e]Calculated using discrete survival analysis.
[f]Calculated using a GEE.

We examined the relationship between STS exposure and implantation failure in this population in two previous studies that utilized different exposure measurement methods from the present study. In an analysis among 921 women who had urine samples available for cotinine measurement, we found that creatinine-adjusted cotinine levels in urine were associated with a slight decrease in first-cycle implantation rates among non-smoking women (11.1% in the lowest cotinine quintile versus 8.2% in the highest quintile; $P = 0.13$; Meeker *et al.*, 2007a). However, in a multivariate analysis, creatinine-adjusted cotinine levels were not associated with failed implantation. Shortly thereafter, in a much larger follow-up study among all non-smoking participants in the study, we found a suggestive association between self-reported STS exposure and failed implantation (Meeker *et al.*, 2007b). We have improved upon our earlier work with our current findings since we believe cotinine in FF is a more biologically relevant exposure measure, as opposed to self-report and urinary concentrations, as it more likely reflects the extent to which the oocyte was directly exposed to the constituents of tobacco smoke during its late development.

We found that, in all analyses for both implantation failure and successful live birth, the OR was farther from the null than the RR. ORs for common outcomes (≥10%) tend to overestimate the relative risk (McNutt *et al.*, 2003). Since implantation failure (53%) and successful live birth (32%) were common outcomes in our study, RRs are likely a

more accurate effect estimate than ORs. However, both ORs and RRs were statistically significant in adjusted models for both implantation failure and successful live birth outcomes, which adds confidence that our results were robust to the type of model used.

Several other studies have explored the relationship between STS exposure and infertility or early pregnancy loss. A Canadian study among 255 women undergoing IVF or ICSI examined differences in implantation and pregnancy rates between smoking groups: those exposed to sidestream (SS) smoke (defined in this study as those who live with a partner that actively smokes), those exposed to mainstream (MS) smoke, the smoke inhaled by the smoker, and NS (Neal *et al.*, 2005). The authors reported that embryo quality was similar between the three groups; however, consistent with our findings, there was a significant difference in implantation rates (MS = 12.0%, SS = 12.6% and NS = 25%; $P < 0.01$) and pregnancy rates (MS = 19.4%, SS = 20.0% and NS = 48.3%; $P < 0.001$) per embryo transfer between groups. Limitations of that study included a small sample size, lack of adjustment for confounding variables and reliance on self-reported exposure. However, despite those limitations, the similarity in results for implantation failure and successful live births between that study and the present study suggests that STS exposure may be detrimental to early pregnancy.

Similar to the present study, Sterzik *et al.* (1996) utilized cotinine levels in FF to examine the effects of STS exposure on fertility and pregnancy among an IVF cohort. They reported no change in pregnancy rates between active, passive and NS. Though not statistically significant, a decrease in fertilization rates was seen among passively exposed subjects (58%) compared with non-smoking subjects (68%). This study's small sample size ($n = 197$; 26 passive smokers) and resultant lack of statistical power may partially explain its null findings. Further, the FF cotinine cut-points used by Sterzik *et al.* (1996) were much higher (NS $\leq$20 ng/ml; passive smoker $>$20 and $\leq$50 ng/ml; active smokers $>$50 ng/ml) than what was used in the present study (NS $\leq$1.11 ng/ml; passive smokers $>$1.11 and $<$10 ng/ml; active smokers $\geq$10 ng/ml). In other words, participants with FF cotinine concentrations as high as 20 ng/ml were considered NS in the Sterzik *et al.* study, but concentrations of that magnitude are more likely to reflect those who actively smoke (Fuentes *et al.*, 2010).

A more recent study investigated associations between paternal smoking and pregnancy loss measured via daily urinary hCG assays among 526 non-smoking Chinese female textile workers, and reported increased odds of early pregnancy loss among women whose husbands smoked more than 20 cigarettes per day (Venners *et al.*, 2004). The results of that study may reflect either effects related to female STS exposure or sperm damage associated with active smoking in males (Calogero *et al.*, 2009), or a combination of both. Another study of fertile women found that the risk of experiencing delayed conception for at least 6 months was significantly elevated among women who reported STS exposure (Hull *et al.*, 2000). The risk estimate for STS exposure was similar in magnitude to that found for women who actively smoked in the study.

Most studies of the mechanisms of early pregnancy difficulties associated with tobacco smoke exposure are focused on active smoking. However, oxidative stress and DNA damage are plausible mechanisms involved in infertility and early pregnancy loss due to the carcinogenic, mutagenic and otherwise toxic constituents of STS. Human studies have found that increased FF cotinine levels are associated with a significant increase in follicular lipid peroxidation intensity (Paszkowski *et al.*, 2002) and an increased risk of DNA damage in granulose-lutein cells (Zenzes *et al.*, 1998). Decreased ovarian function and decreased number and quality of oocytes among smokers versus NS have also been reported (Zenzes *et al.*, 1995; Van Voorhis *et al.*, 1996). An early animal study of the effects of cadmium (a component of tobacco smoke) on reproduction reported that exposure resulted in an increased proportion of oocytes and embryos with chromosomal abnormalities and a decline in the number of oocytes reaching metaphase II (Watanabe *et al.*, 1979). Cadmium may also contribute to placental necrosis, slow trophoblastic development and suppressed steroid biosynthesis and transfer of nutrient metals across the placenta; all of which may contribute to implantation failure and early pregnancy loss (Thompson and Bannigan, 2008).

Neal *et al.* (2008) reported that women exposed to MS smoke had significantly higher levels of benzo[*a*]pyrene (B[*a*]P) in their FF compared with those exposed to SS smoke and NS. They also reported a significant increase in FF B[*a*]P among women who did not conceive compared with those who achieved pregnancy. Since B[*a*]P may mediate the loss of cell adhesion molecules (Shiverick and Salafia, 1999), it may play an important role in embryo implantation failure by altering uterine receptivity. Another study reported that pregnancy rates remain lower for active smokers, despite transferring high-quality embryos during IVF treatment (Ben-Haroush *et al.*, 2011), which indicates that the endometrium plays an important mechanistic role in IVF failure among smokers. The same study, however, found that transferring high-quality embryos maintained high pregnancy rates among passive smokers, and Neal *et al.* (2008) found no significant difference in FF B[*a*]P concentrations between those exposed to SS smoke and NS. Thus, it appears that the mechanisms involved in implantation failure among STS-exposed NS may differ from active smokers.

Because the present study only included couples undergoing IVF, the generalizability of our findings may be limited. Demographic characteristics of an IVF cohort are likely different from the general population. For example, IVF patients tend to be of a higher socioeconomic status due to the cost of treatment, and smoking rates and STS exposure may vary by socioeconomic status. If socioeconomic groups respond differently from STS exposure, this could limit our generalizability. Also, infertile couples' gametes may be more sensitive to STS exposure. Another reason our results may have limited generalizability is because the IVF treatment process does not represent what occurs in natural pregnancy. For instance, fertilization occurs in a laboratory and only the best embryos are selected for transfer. In other words, our results would only be generalizable to similar populations if these conditions are associated with a differential response to STS exposure. However, there is no evidence to date that these factors are associated with differential sensitivity to STS exposure. In addition, implantation failure is not observable in other study designs conducted among the general population which typically rely on estimates of time-to-pregnancy.

The present study is the largest to date on the effects of STS exposure, estimated through an objective biomarker, on fertility or early pregnancy among IVF patients. Thus, we may have been able to observe associations between exposure and outcome that similar, smaller studies would be underpowered to detect. In a previous study, we observed fair to poor reliability (intraclass correlation

coefficient = 0.42–0.52) of FF cotinine concentrations over time (Benedict *et al*., 2011). Since a single FF cotinine measure is only somewhat reliable when evaluating longer-term exposure, and since we were able to leverage longitudinal data from women who underwent multiple treatments cycles, the present results may more accurately represent the effect of STS exposure on implantation failure and live birth success than those from other studies that relied on only first-cycle IVF data.

We hypothesize that cotinine in FF may be a more biologically relevant marker of STS exposure versus cotinine in urine or serum because it represents the developing oocytes' direct exposure to the constituents of tobacco smoke. When comparing FF and urinary cotinine concentrations from the same non-smoking participants from our previous analysis (Meeker *et al*., 2007a), we found that the measures were weakly correlated with one another (Benedict *et al*., 2011). Self-reported STS exposure was also poorly predictive of FF cotinine concentrations in this cohort, possibly because many people are unaware of or underreport their exposure. Thus, the present study may have been less susceptible to exposure misclassification compared with studies relying on urinary cotinine or other markers of STS exposure.

In conclusion, we found a significant increase in the risk of implantation failure following IVF among women exposed to STS compared with those who were unexposed based on cotinine concentrations measured in the FF of the women at oocyte retrieval from repeated treatment cycles. We also observed a significant decrease in the odds of achieving a successful live birth among STS-exposed women. These findings are likely of great public health significance due to continued widespread STS exposure worldwide.

Authors' roles

M.D.B. was responsible for the data analysis, literature review and manuscript preparation. S.A.M. contributed to the study design, methods and data interpretation. A.V. contributed to the statistical approach and critical discussion. K.F.B. also contributed to the statistical approach as well as data analysis. A.F.V. was responsible for data and sample management and data analysis. D.W.C. was responsible for the original cohort study design and execution as well as critical discussion. J.D.M. was responsible for the exposure study design, oversight of sample and data analysis and data interpretation, and manuscript preparation.

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