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Female cigarette smoking and successful fertility treatment: a Danish cohort study

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ABSTRACT

Introduction: Despite smoking being a well-established risk factor for adverse pregnancy and neonatal outcomes a substantial proportion of women of reproductive age smoke. Previously, meta-analyses have indicated a significantly negative impact of female smoking on outcomes of assisted reproduction, yet the majority of the included studies have several, essential methodological limitations. We aimed to investigate whether female cigarette smoking may affect the chance of achieving a clinical pregnancy and live birth among women and couples receiving medically assisted reproduction treatment. **Material and methods:** A cohort study with longitudinally and repeatedly collected exposure information from 1 January 2010 to 31 August 2015, including data on 1708 women and potential partners initiating either intrauterine insemination, in vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) or frozen embryo transfer treatment cycles at the public Fertility Clinic, Aarhus University Hospital, Denmark. Smoking was assessed from self-reported questionnaires completed before treatment. Outcomes were a clinical pregnancy and a live birth. Information on these was obtained from the Danish national health registries, allowing complete follow-up. To evaluate associations between female occasional/daily cigarette smoking and successful medically assisted reproduction treatments a modified Poisson regression with robust standard errors was used. **Results:** Female occasional/daily cigarette smoking was not associated with the chance of achieving a clinical pregnancy or a live birth in all intrauterine insemination or IVF/ICSI treatment cycles. When compared to non-smokers, the adjusted relative risk for obtaining a live birth for those reporting smoking was 1.22 (0.70 - 2.12) among women initiating 1456 intrauterine insemination treatment cycles. Among women initiating 2788 IVF/ICSI treatment cycles, those reporting occasional/daily smoking had a relative risk for obtaining a live birth of 1.15 (0.82 - 1.60) when compared to non-smokers. **Conclusions:** Female occasional/daily cigarette smoking was not associated with the chance of achieving a clinical pregnancy or a live birth when receiving medically assisted reproduction treatments. However, tobacco use before and during pregnancy remains a major cause of reduced fertility as well as maternal, fetal, and infant morbidity and mortality and should strongly be discouraged.

Keywords

Cigarette smoking / Lifestyle / Infertility / Assisted reproduction / Intrauterine insemination / pregnancy

Abbreviations

ART	assisted reproductive technology
FET	frozen embryo transfer
ICSI	intracytoplasmic sperm injection
IUI	intrauterine insemination
IVF	in vitro fertilization
MAR	medically assisted reproduction

Key message:

The results indicate that female occasional/daily cigarette smoking did not influence the chance of achieving a clinical pregnancy or a live birth when receiving medically assisted reproduction treatments.

INTRODUCTION

Despite cigarette smoking being a well-established risk factor for adverse pregnancy outcomes including low birth weight, preterm birth and perinatal mortality, a substantial proportion of women of reproductive age smoke.[1, 2] In Western countries such as Denmark and the United States, every fifth woman smoke and, worryingly, smoking rates have increased in recent years in young Danes about to enter the reproductive age.[3,4] In addition to known risks during pregnancy, smoking has been associated with impaired reproductive function, including decreased fecundity among couples conceiving spontaneously.[5] Smoking may impact female fertility through various mechanisms including gametogenesis, oocyte depletion, follicular growth, embryo transport, endometrial receptivity and changes in hormonal levels.[6]

Infertility is a major public health concern affecting up to 15-26% of all Western couples.[7-9] In recent decades, an increasing number of fertility treatments have been performed every year.[10] Yet, improvements in success rates per initiated cycle remain relatively small. The American Society for Reproductive Medicine stated in 2018 that the literature strongly supports an association between cigarette smoking and female infertility.[11] Despite numerous publications on this subject, many previous studies have, however, several, essential limitations, including small sample sizes and selected study populations. Furthermore, they are restricted to couples receiving assisted reproductive technology (ART) treatment and based only on one measurement of exposure, thus not accounting for possible variation in smoking during consecutive treatment cycles. Finally, the vast majority have failed to account for important confounding factors such as the incomparable effect of female age. Thus, updated and methodologically well-conducted studies are warranted for clinicians to provide guidance.

In this cohort study of women initiating medically assisted reproduction (MAR) treatment, we investigated the association between female occasional/daily cigarette smoking and successful treatment.

MATERIAL AND METHODS

Study population

The Aarhus MAR-cohort include data on Danish women and potential partners initiating fertility treatment at the public fertility clinic, Aarhus University Hospital in Denmark from 1 January 2010 until 31 August 2015. In the study period, women/couples initiated from one to a maximum of 13 treatment cycles. The database included information from questionnaires and treatment charts. The eligibility criteria for initiating fertility treatment and inclusion in this study were 1) Danish Civil Registration (CPR) number 2) age below 41 years, 3) single and childless or in a relationship with a man or a woman with no common children and 4) initiation of one of the following treatment modalities: intrauterine insemination (IUI) with either partner/homologous semen (IUI-H) or donor semen (IUI-D), in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) or frozen embryo transfer (FET).[12]

Before the first consultation, all women and potential partners were asked to complete an obligatory baseline questionnaire on lifestyle factors. In October 2011, the baseline questionnaire was updated to further include questions on exposure to any passive smoking, time until the first cigarette in the morning and the use of nicotine replacement therapy. Before each treatment cycle, women/couples were asked to complete an additional and shorter cycle-specific-questionnaire on lifestyle factors and for every treatment cycle, medical doctors completed a treatment chart.

We collected data on 1872 women/couples; after exclusions, 1708 of these were included in this study (Supporting information Figure S1).

Ascertainment of exposure

Self-reported information on smoking status and number of cigarettes smoked per day (cig./day) was obtained from the baseline questionnaire and the subsequent cycle-specific questionnaires. In the questionnaires, women were asked whether they smoked (yes; no). There was no information on smoking history. Thus, the category of 'smokers' consist of occasional and daily smokers and the category of 'non-smokers' consist of never and ex-smokers. Smoking women were further asked to report the number of cigarettes smoked per day in predefined categories (0-5; 6-10; 11-15; 16-20 or >20 cig./day).

Ascertainment of outcome

Outcomes were defined per initiated treatment cycle as either: a clinical pregnancy (any viable intrauterine pregnancy confirmed by ultrasonography performed in pregnancy weeks 7-8 (yes; no)) recorded in the Danish in vitro fertilization (IVF) register or a live born child (yes; no) registered in the Danish medical birth register (MBR). We considered it a live birth of a given treatment cycle, if at least one live born child was registered within 140-308 days (20-44 completed weeks) from treatment initiation.[13, 14]

Treatment cycle number was defined based on information on all MAR-treatment cycles obtained from the Danish IVF register containing information on all treatments performed at public and private fertility clinics in Denmark.

Ascertainment of confounders

Information on potential confounders was available from the Aarhus MAR cohort, the IVF register, the Danish MBR register, the Danish national patient register, and the population education register at Statistics Denmark.[15, 16] Based on previous literature and by using Directed Acyclic Graphs (DAGs), potential confounders were identified *a priori* (Supporting information Figure S2).[17] The following variables were included in the analyses: age at treatment initiation (years); objectively measured body mass index (kg/m^2), self-reported alcohol intake (drinks/week), self-reported coffee consumption (0; 1-5 cups/day) and from register data chronic diseases (hyperthyroidism and hypothyroidism; diabetes mellitus type 1; eating disorders; hyperprolactinemia; chronic renal failure; Turner syndrome; Addison's disease; coeliac disease (no; yes)), and highest attained educational level (0-10; 11-14 and >14 years duration). The variables parity (nulliparous; multiparous) and a potential male partner's self-reported tobacco consumption (cig./day) were included in sub-analyses (available for 70-76%).

Statistical Analyses

We had complete information on lifestyle factors (smoking, BMI, coffee and alcohol) from the baseline questionnaire on 95% of the study population. Follow-up data on lifestyle was collected on 70% of IUI patients and 73% of IVF/ICSI patients when initiating the first treatment cycle.

Before initiating a second or third treatment cycle, follow-up data was collected on 46% of IUI patients and 51% of IVF/ICSI patients and on 41% and 51%, respectively. Data on educational level was complete for 99% of patients receiving IUI and for 98% of those receiving IVF/ICSI. Data on chronic diseases and parity was available for all.

Multiple imputation using chained equations was applied to handle missing data in this study.[18] The imputation model was based on data from the baseline questionnaire, the cycle-specific lifestyle questionnaires, and the register-based data. A total of 100 datasets (m=100) were imputed. Because of too little variance in the observed data, we were underpowered to fit an imputation procedure in some of the sub-analyses. To account for missing data in these analyses, the single-value imputation model of "last value carried forward" was applied. For details on missing data, possible missing mechanisms, considerations on challenges and the imputation model applied, see Supporting Information Appendix S1.

Data analyses

In the main analyses, a modified Poisson regression with robust standard errors was performed to estimate the associations between female exposure to daily cigarette smoking and a successful outcome following all consecutive treatment cycles.[19] The analyses were stratified on treatment modality (ie IUI and IVF/ICSI), adjusted for the potential confounders mentioned above, and the cycle number was included as a categorical covariate in both crude and adjusted analyses. By including robust standard errors, the model accounted for any correlation that may have arised if the same woman contributed with repeated treatment cycles. We present estimates as crude and adjusted relative risks (RR) with 95% confidence intervals (CIs) for achieving a clinical pregnancy or a live birth.

Analyses restricted to the first initiated treatment cycle (IUI; IVF/ICSI; FET) were performed. Also, cycle-specific associations were estimated for those receiving a second or third IUI or IVF/ICSI treatment cycle.

Further, we assessed the crude cumulative chance of live birth after three consecutive IUI or IVF/ICSI cycles, including all frozen embryo transfers resulting from egg collection in the fresh cycles. The comparison between groups was done by bootstrapping. All data management, imputation and analyses were carried out using STATA 15 (Statacorp, College Station, Texas, USA).

Sub-analyses and sensitivity analyses

Sub-analyses included 1) stratification by IVF and ICSI, 2) stratification of the IVF/ICSI analysis according to number of embryos transferred (one vs. two embryos), 3) restriction to nulliparous women, 4) restriction to those who managed to complete a given treatment cycle, 5) restriction to heterosexual couples using homologous semen with further adjustment for male tobacco consumption, 6) restriction to infertile heterosexual couples, and 7) performing the main analyses with the most parsimonious adjustment model including female age, educational level and treatment cycle number, considering the relatively small proportion of smokers across cycles.

In sensitivity analyses we 1) excluded the few observations reporting either exposure to passive smoking or use of nicotine replacement therapy, as this information was only assessed among those receiving the updated edition of the baseline questionnaire and 2) assessed the robustness of the multiple imputation procedure applied to account for missing data (first, imputing a lower number of datasets (m=50); second, restriction to complete case analyses; third, using single value imputation by last value carried forward).

Ethical approval

The study was approved by the Danish Data Protection Agency (Aarhus University no. 2016-051-000001, unique AU-id 783) and the Danish Health Authority (Case no. 3-3013-1023/1/) and all participants provided written informed consent before enrolment. According to the Danish Ethical Review system, ethical approval was not needed. In agreement with all Danish rules and regulations for research, data could not be reported for any given variable with less than five observations, including information on missing data.

RESULTS

A total of 1708 women and potential partners were included in this cohort. At baseline, approximately 14% of all included women reported occasional or daily smoking with 6% smoking 0-5 cig./day, 4% smoking 6-10 cig./day, 3% smoking 11-15 cig./day, and 1% smoking 16 cig./day or more. When initiating the first IUI treatment cycle, approximately 6% had stopped smoking and no one had started smoking with 4.5% smoking 1-5 cig./day and 4.5% smoking more than 5

cig./day. The proportion of smokers decreased further among those initiating the second IUI cycle with 4% smoking 1-5 cig./day and 3% smoking more than 5 cig./day.. Among women initiating the first IVF/ICSI treatment cycle, approximately 7.5% stopped smoking while 0.5% had started smoking with approximately 5% smoking 1-5 cig./day and 2% smoking more than 5 cig./day. When initiating the second IVF/ICSI cycle the proportion remained fairly constant with 4% smoking 1-5 cig./day and 3% smoking more than 5 cig./day.

In the total cohort, women who smoked occasional or daily were more likely to drink alcohol, have lower educational level, have a male partner who smoked and have been trying to conceive for a longer time as compared to non-smoking women (Table 1 and 2).

Compared to non-smokers, the adjusted relative risks for achieving a live birth among those reporting occasional/daily smoking were 1.22 (0.70 - 2.12) among women initiating 1456 IUI treatment cycles, and 1.15 (0.82 - 1.60) among women initiating 2788 IVF/ICSI treatment cycles (Table 3). When restricting the analyses to the first treatment cycle (Table 4) results were similar in magnitude and direction. Yet, the magnitude became less pronounced or the direction changed in most circumstances when restricting the population to the limited number initiating a second or a third treatment cycle. However, the 95% CIs were wide and despite the change in direction, the estimates remained close to one (Supporting Information Table S1 and Table S2).

The cumulative probability of live birth for three consecutive IUI or IVF/ICSI treatment cycles did not differ significantly, yet a tendency towards lower success rates among smoking women was observed for IUI treatments (Supporting information Table S3). In sub-analyses, the investigated associations were comparable to the results reported in the main analyses (Supporting Information Tables S4).

Further, excluding the few women exposed to passive smoking or use of nicotine substitutes in sensitivity analyses did not alter the results. When assessing the robustness of the applied multiple imputation procedure neither varying the number of imputations, performing complete case analyses nor applying the single-value imputation procedure of last value carried forward altered the results considerably (Supporting Information Tables S5).

DISCUSSION

In this large cohort study, we found no indication of an association between female smoking at treatment initiation and the chance of achieving a clinical pregnancy or a live birth following different MAR treatments. Overall, estimates were of small magnitude and no systematic pattern was observed for the association with results deviating around the null-effect. However, we were not able to discriminate between occasional and daily smokers and self-reported tobacco consumption was low.

A review of the literature revealed that only one study addressed the association between smoking and treatment outcomes among women receiving IUI. In accordance with our results, Farhi and Orvieto found no statistically significant difference in pregnancy rates among 885 Israeli women undergoing 1-4 IUI cycles, yet they indicated a higher gonadotropin dosage among smokers.[20] However, exposure was only assessed once and more importantly, the study did not adjust for any potentially important confounders, leaving room for biased results.

In contrast, the association among women undergoing ART treatment has been studied extensively during the last decades, as reviewed by Waylen et al. and Zhang et al., the latter including 28 studies.[21, 22] Despite the comprehensive amount of existing literature, several serious methodological limitations hinder any firm conclusions and call for further attention. General limitations include small sample sizes, heterogeneous study populations and exposure only being assessed once. Many previous studies are prone to selection bias with few providing information on the recruitment process or participation rate and many condition the analyses to cycles with ET. Also, most previous studies have been restricted to couples receiving ART and have reported on multiple outcomes, yet across studies smoking has not consistently been associated with the same endpoints, and the role of chance is rarely addressed by adjustment for multiple testing. Further, the vast majority of the studies present only p-values from Student's t-test or chi-square test while not accounting for female age, which may be considered the strongest predictive factor for a successful fertility treatment.[23] Therefore, reliance on unadjusted estimates is considered a crucial limitation that questions the validity of many previous results.

Our results of no strong, if any, association are in agreement with the most recent study on occasional/daily smokers by Rockhill et al., a large nationwide U.S. study covering 98%

of all ART procedures performed between 2009 and 2013.[24] Unfortunately, the study was unable to report on IUI treatments, analyses on treatment outcomes were restricted to cycles with embryo transfer and they did not have information on potential confounders such as male smoking status or education. Further, in the U.S. setting, smokers are excluded from receiving ART treatment coverage, and the study is most likely prone to misclassification of exposure due to nondisclosure of smoking status.[1, 25] As information on smoking status was assessed without knowledge of the treatment outcome, this would probably attenuate any true association towards the null.

Among major strengths of this study were the large infertility cohort from a public fertility clinic and the comprehensive longitudinal data collection on cycle-specific exposure on different MAR treatments including IUIs. All patients treated in the study period were included. Further, by including all initiated treatment cycles, we were able to include early treatment failures possibly associated with the exposure, thus limiting the risk of selection bias.[26] The study population is heterogeneous and among single women and lesbians, a higher proportion was smokers, yet results remained the same when restricting to infertile heterosexual couples. Further, updated data on lifestyle factors were only collected on a varying sub-population beyond the first treatment cycle. Thus, as complete case analyses may be prone to selection bias, we applied multiple imputation to handle missing data (Supporting information Appendix S1).

Cigarette smoking was self-reported and exposure misclassification is a source of bias to consider. Based on the available data, we were unable to distinguish between ex-smokers and never-smokers in the group of non-smoking women and to distinguish occasional smokers from daily smokers in the group of smoking women. Thus, an important study limitation is the inability to investigate a potential effect among purely daily smokers as compared to never-smokers. It would have been interesting to investigate if there was a difference in pregnancy rate and live birth rate between light and heavy smokers. Unfortunately, the study was underpowered to categorize the exposure in further categories than the two presented. Also, tobacco consumption was measured in pre-defined categories, not in actual number of cigarettes smoked daily. At baseline, the largest proportion of smoking women (46%) was in the category who reported smoking 0 to 5 cig./day. Consequently, the inclusion of occasional smokers in the lowest exposure category could dilute a potential effect of smoking on treatment. Also, the self-reported tobacco consumption was very low, compared with the general population.[27] This might partly be due to

under-reporting, a social desirability bias. A considerable proportion of women reported stopping smoking before initiating the first treatment cycle and were categorized as non-smokers in the first treatment cycle. However, we were unable to further stratify into a separate exposure group of recent smokers. Further, given the low prevalence of self-reported tobacco consumption in this population the study may have been underpowered to detect any differences in MAR outcomes between smokers and non-smokers. Although collecting lifestyle data for each consecutive treatment cycle, exposure was only reported before initiating a given cycle, hence we were unable to assess possible exposure fluctuations during the course of a treatment cycle. In a previous study, smoking women spontaneously reduced cigarette smoking from treatment initiation to oocyte retrieval.[28] Therefore, if a woman classified as a smoker in this study quit smoking during treatment, this could bias the results and possibly explain a seemingly positive association, if smoking cessation immediately impacts on success rate. Yet, even though underreporting of smoking cannot be ruled out, the misclassification would most likely be non-differential, as the exposure was assessed prior to treatment initiation and thus without knowledge of the outcome. No biomarker of nicotine was measured within this cohort and data did not allow us to take the cumulative amount of exposure (pack years) into account. Yet, among infertile women self-report of smoking status has been reported a valid measure when compared to serum cotinine levels. [29]

By having access to all relevant patient records and by linkage to nationwide Danish health care registries, follow-up was complete: the achievement of a clinical pregnancy was verified by ultrasonography by a specialist gynaecologist, and complete registrations on a live birth was available in the Danish health care registries. We had the ability to adjust for a variety of potentially important confounders including male cigarette smoking, still residual confounding from misclassifications of covariates and unmeasured confounders should be considered.

Recognizing the deleterious effects of smoking on pregnancy and neonatal outcomes, clinicians should still urge women to stop smoking before initiating fertility treatment.[2] Given that the infertile population might be more receptive to smoking cessation interventions prior to pregnancy and that one-third to one-half continue to smoke during pregnancy the recommendation of smoking cessation is essential, despite of our findings of no association between occasional/daily smoking and success of MAR treatments.[30, 31]

CONCLUSION

The results of this study did not indicate an association between female cigarette occasional/daily smoking and the chance of achieving a clinical pregnancy or a live birth among women and couples undergoing MAR treatments. However, the role of non-differential exposure misclassification cannot be ruled out. Despite these results, the literature regarding pregnancy and neonatal outcomes consistently report significant and harmful effects among smoking mothers. Thus, given the obligation fertility doctors have for the established pregnancy and the welfare of children born following fertility treatment the best clinical advice is still not to smoke during the course of fertility treatment.

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LEGENDS FOR SUPPORTING INFORMATION

FIGURE S1. Flowchart for the study population in the Aarhus MAR cohort

FIGURE S2. Directed Acyclic Graph (DAG)

TABLE S1. Associations between female smoking status and a successful intrauterine insemination treatment cycle (Restricted to a given treatment cycles, relative risk (RR))

TABLE S2. Associations between female smoking status and a successful IVF/ICSI treatment cycle, (Restricted to a given treatment cycles, relative risk (RR))

TABLE S3. Cumulative chance of live birth for three consecutive treatment cycles (crude estimates) by smoking status

TABLES S4. Results from sub-analyses

TABLES S5. Results from sensitivity analyses

APPENDIX S1. Missing data and Multiple Imputation

Table 1. Characteristics of the study population by smoking status during the first intrauterine insemination (IUI) treatment cycle, including 437 women and based on multiple imputations*.

	Smoking status		
	Non-smokers (never and ex-smokers)	**Smokers (Occasional and daily smokers)**	**Total**
Women (%)	90.5	9.5	100
Age at start of treatment (mean years (SD))	31.5 (4.3)	31.0 (4.8)	31.4 (4.3)
Marital status (%)			
Male partner	77.4	69.3	76.7
Female partner	6.9	6.2	6.9
Single	15.6	25.5	16.5
Parity (nulliparous (%))	99.5	100	99.5
Time trying to conceive at baseline (mean months (SD))	20.9 (12.8)	28.2 (25.7)	21.5 (14.5)
Intercourse (weekly frequency) (mean (SD))	2.5 (1.1)	2.8 (1.1)	2.5 (1.1)
Cancelled treatment cycle (yes (%))	11.0	8.3	10.8
BMI (mean (SD))	24.0 (4.4)	25.2 (4.0)	24.2 (4.4)
Alcohol (drinks/week) (mean (SD))	1.9 (2.5)	2.3 (2.7)	1.9 (2.5)
Coffee (cups/day) (%)			
0	42.3	43.7	42.4
1-5	57.7	56.3	57.6
Aetiology of infertility (%)			
Female factor [a]	18.1	22.9	18.6
Male factor	24.1	16.4	23.4
Female and male factor	2.9	6.6	3.2
Single or lesbian	21.4	30.7	22.3
Unexplained	33.5	23.4	32.6
Highest completed education [b] (%)			
Low	4.9	9.0	5.3
Middle	25.5	59.2	28.7
High	69.6	31.8	66.0
Chronic diseases [c] (yes (%))	5.1	1.7	4.8
Male cigarette smoking status			
Non-smoker (%)	83.9	57.1	81.5

Smoker (%)	16.1	42.9	18.5

* Study characteristics are based on the imputed distribution and therefore presented as percentage or mean (SD)

[a] Female factor includes: tubal factor, ovarian factor including PCOS, endometriosis

[b] Highest completed education: Low (0-10 years duration); Medium (11-14 years duration); High (≥ 14 years duration)

[c] Chronic diseases includes: Hyperthyroidism and Hypothyroidism; Diabetes Mellitus type 1, Eating disorders; Hyperprolactinaemia; Chronic renal failure; Turner syndrome, Addison's disease; Coeliac disease

Table 2. Characteristics of the study population by daily tobacco consumption during the first in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment cycle, including 1421 women and based on multiple imputations*.

	Smoking status		
	Non-smokers (never and ex-smokers)	**Smokers (Occasional and daily smokers)**	**Total**
Women (%)	92.6	7.4	100
Age at start of treatment (mean years (SD))	31.9 (4.6)	32.1 (5.0)	31.9 (4.6)
Marital status (%)			
Male partner	91.0	93.9	91.2
Female partner	1.6	2.6	1.7
Single	7.4	3.5	7.1
Parity (nulliparous (%))	98.6	94.0	98.2
Time trying to conceive at baseline (mean months (SD))	22.7 (14.8)	27.9 (18.8)	23.1 (15.2)
Intercourse (weekly frequency) (mean (SD))	2.6 (1.4)	2.9 (2.0)	2.6 (1.5)
Cancelled treatment cycle (yes (%))	19.3	16.8	19.1
BMI (mean (SD))	24.3 (4.7)	25.5 (5.0)	24.4 (4.7)
Alcohol (mean (SD))	1.6 (2.1)	2.0 (2.5)	1.6 (2.2)
Coffee (cups/day) (%)			
0	45.6	38.6	45.1
1-5	54.4	61.4	54.9
Aetiology of infertility (%)			
Female factor [a]	20.2	19.4	20.1
Male factor	46.9	44.0	46.7
Female and male factor	7.9	9.6	8.1
Single or lesbian	7.5	5.4	7.3
Unexplained	17.5	21.7	17.8
Highest completed education [b] (%)			
Low	5.4	16.0	6.2
Middle	27.2	44.1	28.4
High	67.4	39.9	65.4
Chronic diseases [c] (yes (%))	7.5	1.7	7.1

Male cigarette smoking status			
Non-smoker (%)	84.6	29.9	80.8
Smoker (%)	15.4	70.1	19.2

* Study characteristics are based on the imputed distribution and therefore presented as percentage or mean (SD)

[a] Female factor includes: tubal factor, ovarian factor including PCOS, endometriosis

[b] Highest completed education: Low (0-10 years duration); Medium (11-14 years duration); High (≥ 14 years duration)

[c] Chronic diseases includes: Hyperthyroidism and Hypothyroidism; Diabetes Mellitus type 1, Eating disorders; Hyperprolactinaemia; Chronic renal failure; Turner syndrome, Addison's disease; Coeliac disease

Table 3. Pooled associations between female smoking status and a successful fertility treatment by type of treatment including 1456 IUI cycles and 2788 IVF/ICSI cycles (all initiated treatment cycles, relative risk (RR)).

	Clinical pregnancy*				**Live birth**			
Smoking status	Total, %[a]	Cases, %[b]	cRR (95%CI)	aRR[d] (95%CI)	Total, %[a]	Cases, %[c]	cRR (95%CI)	aRR[d] (95%CI)
			IUI				**IUI**	
Non-smoker (ex- and never smokers)	92.5	13.7	1.00 (Reference)	1.00 (Reference)	92.5	12.5	1.00 (Reference)	1.00 (Reference)
Smokers (occasional- and daily smokers)	7.5	16.0	1.18 (0.69 - 2.02)	1.06 (0.60 - 1.87)	7.5	16.6	1.35 (0.80 - 2.28)	1.22 (0.70 - 2.12)
			IVF + ICSI				**IVF + ICSI**	
Non-smoker (ex- and never smokers)	93.3	26.4	1.00 (Reference)	1.00 (Reference)	93.3	24.3	1.00 (Reference)	1.00 (Reference)
Smokers (occasional- and daily smokers)	6.7	27.5	1.04 (0.76 - 1.41)	1.17 (0.85 - 1.60)	6.7	24.4	1.00 (0.72 - 1.39)	1.15 (0.82 - 1.60)

Intrauterine insemination (IUI); In vitro fertilization (IVF); Intracytoplasmic sperm injection (ICSI); crude relative risk (cRR); adjusted relative risk (aRR); confidence interval (CI)

* Live and intrauterine pregnancy visualized by ultrasound performed at pregnancy weeks 7-8

[a] Distribution of the study populations in each category of smoking status, based on multiple imputation

[b] Percentage of cycles resulting in a clinical pregnancy in each category of smoking status, based on multiple imputation

[c] Percentage of cycles resulting in a live birth in each category of smoking status, based on multiple imputation

[d] Adjusted for: female age, BMI, daily coffee consumption, weekly alcohol consumption, chronic diseases, education and treatment cycle number

Table 4. Associations between female smoking status and a successful fertility treatment by type of treatment including 437 IUI cycles, 1421 IVF/ICSI cycles and 644 FET cycles (first initiated treatment cycle, relative risk (RR)).

	Clinical pregnancy*				Live birth			
Smoking status	Total, %[a]	Cases, %[b]	cRR (95%CI)	aRR[d] (95%CI)	Total, %[a]	Cases, %[c]	cRR (95%CI)	aRR[d] (95%CI)
			IUI				**IUI**	
Non-smoker (ex- and never smokers)	90.5	15.0	1.00 (Reference)	1.00 (Reference)	90.5	13.3	1.00 (Reference)	1.00 (Reference)
Smokers (occasional- and daily smokers)	9.5	18.3	1.22 (0.56 - 2.66)	1.12 (0.50 - 2.53)	9.5	18.3	1.38 (0.63 - 3.04)	1.22 (0.54 - 2.77)
			IVF + ICSI				**IVF + ICSI**	
Non-smoker (ex- and never smokers)	92.6	28.0	1.00 (Reference)	1.00 (Reference)	92.6	25.6	1.00 (Reference)	1.00 (Reference)
Smokers (occasional- and daily smokers)	7.4	33.4	1.19 (0.83 - 1.72)	1.32 (0.90 - 1.91)	7.4	28.7	1.12 (0.75 - 1.66)	1.25 (0.84 - 1.88)
			FET				**FET**	
Non-smoker (ex- and never smokers)	94.8	22.2	1.00 (Reference)	1.00 (Reference)	94.8	20.0	1.00 (Reference)	1.00 (Reference)
Smokers (occasional- and daily smokers)	5.2	13.6	0.56 (0.15 - 2.05)	0.55 (0.15 - 2.05)	5.2	14.4	0.66 (0.18 - 2.36)	0.64 (0.18 - 2.34)

Intrauterine insemination (IUI); In vitro fertilization (IVF); Intracytoplasmic sperm injection (ICSI); Frozen embryo transfer (FET); crude relative risk (cRR); adjusted relative risk (aRR); confidence interval (CI)

* Live and intrauterine pregnancy visualized by ultrasound performed at pregnancy weeks 7-8

[a] Distribution of the study populations in each category of smoking status, based on multiple imputation

[b] Percentage of cycles resulting in a clinical pregnancy in each category of smoking status, based on multiple imputation

[c] Percentage of cycles resulting in a live birth in each category of smoking status, based on multiple imputation

[d] Adjusted for: female age, BMI, daily coffee consumption, weekly alcohol consumption, chronic diseases, education