Received: 26 November 2019 | Revised: 2 March 2020 | Accepted: 5 March 2020

DOI: 10.1111/andr.12782

ORIGINAL ARTICLE

ANDROLOGY | WILEY

Fetal exposure to paternal smoking and semen quality in the adult son

Katia Keglberg Hærvig[1,2] | Birgit Bjerre Høyer[1,3] | Aleksander Giwercman[4] | Karin Sørig Hougaard[2,5] | Cecilia Høst Ramlau-Hansen[3] | Ina Olmer Specht[6] | Gunnar Toft[7] | Jens Peter Bonde[1,2] | Sandra Søgaard Tøttenborg[1]

[1]Department of Occupational and Environmental Medicine, Bispebjerg & Frederiksberg Hospital, Copenhagen, Denmark

[2]Department of Public Health, The Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

[3]Department of Public Health, Research Unit for Epidemiology, Aarhus University, Aarhus, Denmark

[4]Department of Translational Medicine, Molecular Reproductive Medicine, Lund University, Lund, Sweden

[5]National Research Centre for the Working Environment, Copenhagen, Denmark

[6]Research Unit for Dietary Studies, The Parker Institute, Bispebjerg Frederiksberg Hospital, Copenhagen, Denmark

[7]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark

Correspondence
Sandra Søgaard Tøttenborg, Department of Occupational and Environmental Medicine, Bispebjerg & Frederiksberg Hospital, Bispebjerg Bakke 23, Building 20F, 2400 Copenhagen NV, Denmark.
Email: sandra.soegaard.toettenborg@regionh.dk

Funding information
This article is part of the ReproUnion collaborative study, co-financed by the European Union, Intereg V ÖKS. The FEPOS project was further funded by the Lundbeck Foundation, the Capital Region of Denmark, Medical Doctor Sofus Carl Emil Friis and Spouse Olga Doris Friis' Grant, Axel Muusfeldt's Foundation and AP Møller Foundation.
The Danish National Birth Cohort was established with a significant grant from the Danish National Research Foundation. Additional support was obtained from the Danish Regional Committees, the Pharmacy Foundation, the Egmont Foundation, the March of Dimes Birth Defects Foundation, the Health Foundation, and other minor grants. The DNBC Biobank has been supported by the Novo Nordisk Foundation and the Lundbeck Foundation. Follow-up of mothers and children have been supported by the Danish Medical Research Council (SSVF 0646, 271-08-0839/06-066023, O602-01042B, 0602-02738B), the Lundbeck Foundation (195/04, R100-A9193), The Innovation Fund

Abstract

Background: The negative impact of maternal smoking during pregnancy on offspring semen quality is well established. Less is known about the impact of paternal smoking.

Methods: We estimated differences in semen parameters and testicle size according to paternal smoking in 772 adult sons of women enrolled in the Danish National Birth Cohort when pregnant. Parents' smoking was reported around gestational week 16, and analyses were adjusted for parents' ages at conception, maternal pre-pregnancy body mass index, maternal alcohol and caffeine intake, family occupational status, ejaculatory abstinence time, clinic of semen analysis, and season.

Results: Sons of smoking fathers and non-smoking mothers had a 10% (95% confidence interval: −24%, 7%) lower semen concentration and 11% (95% confidence interval: −27%, 8%) lower sperm count than sons of non-smoking parents. Having two smoking parents was associated with 19% reduction in sperm count (95% confidence interval: −37%, 3%). Paternal smoking was not associated with volume, motility, or morphology. Adjusting for maternal smoking, paternal smoking was associated with a 26% increased risk of small testicular volume (95% confidence interval: 0.89, 1.78).

Discussion: Exclusion of sons with a history of testicular cancer, chemotherapy, orchiectomy, and with only one or no testicles may have caused us to underestimate associations if these men's reproductive health including semen quality are in fact more sensitive to paternal smoking.

Conclusion: The study provides limited support for slightly lower sperm concentration and total sperm concentration in sons of smoking fathers, but findings are also compatible with no association.

Denmark 0603-00294B (09-067124), the Nordea Foundation (02-2013-2014), Aarhus Ideas (AU R9-A959-13-S804), University of Copenhagen Strategic Grant (IFSV 2012), and the Danish Council for Independent Research (DFF – 4183-00594 and DFF – 4183-00152).

KEYWORDS

infertility, fetal programming, paternal exposure, sperm count

1 | INTRODUCTION

Infertility is the most prevalent chronic disorder among people of reproductive age affecting up to 15% of couples.[1] Low semen quality is a contributing factor in up to half of these cases.[2] Little is known about the causes of low semen quality, but exposure to maternal smoking during fetal life has repeatedly been associated with reduced semen quality in adult men[3,4]—possibly because of disturbances of the intrauterine hormonal environment during the critical time of gonadal development during first trimester.[5]

Evidence is growing that also paternal exposure to tobacco smoke can cause intergenerational effects through the germ line. A systematic review including more than 200 studies found strong evidence of substantially higher rates of DNA damage, mutations, and chromosomal aberrations in spermatozoa of smokers.[6] Yet, only a handful studies have evaluated the association with paternal smoking and semen quality or risk of oligospermia. While the majority found no association,[7-10] a Swedish research group detected a 46% (95% confidence interval (CI) 21%-64%) lower total sperm count and 35% (95% CI 8.1%-55%) lower sperm concentration in paternally but not maternally exposed men.[11] These findings were only slightly attenuated in a follow-up study of 104 of the sons for whom it was possible to adjust for maternal cotinine from stored blood samples.[3] In most of the studies, information was collected retrospectively from either the sons or the mothers making it prone to recall bias.[3,7-8,10,11] Moreover, most study populations were small,[3,7,9-11] and most studies lacked information on related parental risk factors during pregnancy to limit potential confounding.

In this large-scale population-based follow-up study, we used detailed information on various markers of semen quality, and fetal exposure to paternal and maternal smoking collected during pregnancy alongside other important prenatal risk factors. The aim was to determine whether paternal smoking, alone or in combination with maternal smoking during pregnancy, is associated with reduced semen quality.

2 | METHODS

2.1 | Study population

We made use of the Fetal Programming of Semen Quality (FEPOS) cohort which is a sub-cohort of sons born to mothers included in the Danish National Birth Cohort (DNBC). The DNBC holds information on approximately 92,000 mothers and their children born during 1996-2003.[12]

To be included in the FEPOS cohort, the sons had to be at least 18 years and 9 months of age, live in or near Copenhagen or Aarhus, and their mothers should as a minimum have provided one blood sample during pregnancy and responded to two computer-assisted telephone interviews around gestational weeks 16 and 30 (n = 19,343). Further, to participate the sons should have both testicles descended in the scrotum, not have undergone sterilization, orchiectomy, or chemotherapy. Using a digitalized and comprehensive recruitment system, eligible sons were consecutively invited to participate in the FEPOS cohort during the study period. An invitation letter was sent to their personal secure digital mailbox "e-Boks" linked to the unique personal identification number. E-Boks is automatically created at the age of 15 years and used for bi-directional communication with public authorities. Upon digitally consenting using their NemID (a common secure login) participants received links to an online questionnaire and booking system, to schedule an appointment for a clinical visit at either the Department of Occupational and Environmental Medicine at Bispebjerg and Frederiksberg Hospital (Copenhagen) or the Department of Occupational Medicine at Aarhus University Hospital (Aarhus). From March 2017 to March 2019, we invited 4,254 young men to answer a comprehensive electronic questionnaire addressing health behaviors, provide a semen sample, and undergo a clinical examination, of which 772 (18%) participated.

2.2 | Parental smoking

Information on parental smoking was derived from a computer-assisted telephone interview conducted during pregnancy at approximately gestational week 16.[12] The women were asked "Have you smoked during pregnancy." If they answered "*yes*," they were asked "Do you smoke now?" (*yes daily*; *yes*, *less than daily*; *no*). The women were also asked whether their husband or partner smoked "Does your husband/partner smoke" (*yes daily*; *yes*, *less than daily*; *no*). Mothers were coded as smokers if they had smoked at any point between conception and interview even if they did not still smoke at the time of the interview. Fathers were coded as smoker if the woman reported him to smoke daily or less than daily.

2.3 | Semen characteristics and testicular volume

The participants were offered to collect the semen sample at the clinic or at home and provided a sample container and were provided detailed instructions on collection, to abstain from ejaculation

48-72 hours, and transportation of the sample. All semen analyses followed the recommendations by the World Health Organization 2010.[13] Semen volume was measured by weighing of the sample in the pre-weighed container. The sample was then placed in a 37°C for liquefaction. After liquefaction, samples were analyzed manually for sperm concentration, total sperm count, and motility (proportion of progressive; non-progressive; and immotile spermatozoa) by a trained medical laboratory technician affiliated to the clinic in Copenhagen and in Aarhus, respectively. Azoospermia was defined by having no spermatozoa in the semen sample. External quality control with the European Society of Human Reproduction and Embryology (ESHRE) External Quality Assessment scheme (Centre for Andrology, Karolinska University Hospital, Stockholm, Sweden) indicated no large systematic differences between the FEPOS laboratory technologists and the expert examiners. Morphology was analyzed at the Reproductive Medicine Centre, Skåne University Hospital, Malmö, Sweden. Testicular volume was measured by the participants themselves during the clinical examination using a Prader Orchidometer. This method has previously been shown valid.[14] Small testicular volume was defined as < 15 mL.[15]

2.4 | Covariates

Information on maternal pre-pregnancy body mass index (BMI), weekly glasses of alcohol, including beer, wine, and spirits, cups of coffee per day, and family occupational status (*high-grade professionals; low-grade professionals; skilled worker; unskilled worker; student; economically inactive*) was obtained from the DNBC interview around gestational week 16. Maternal and paternal age at birth was obtained from the Danish Medical Birth Registry.[16] Place of son's clinical visit (*Aarhus; Copenhagen*) was used to correct for potential differences between laboratories, recruitment procedures and participants. Information on the adult sons was gained from the electronic questionnaire filled out prior to the examination and included alcohol consumption and smoking habits. Information on urogenital malformations (torsion of the spermatic cord, varicocele, hydrocele or phimosis, or any of the following International Classification of Disease (ICD)-10 codes: Q53, Q54, Q55, Q56) and congenital malformations (cryptorchidism or hypospadias or any of the following ICD-10 codes: Q53, Q54, Q55, Q56) were obtained from the FEPOS questionnaire and the Danish National Patient Registry.[17] BMI, days since last ejaculation, minutes from ejaculation to start of semen analysis, season for delivery of the semen sample, spillage of semen sample, and place of ejaculation (*clinic; home*) were recorded at the clinical visit.

2.5 | Statistical method

Baseline characteristics according to parental smoking status during pregnancy were presented as proportions or means with standard deviations (SD). We used negative binomial regression analyses to calculate the association between parental smoking and semen volume, sperm concentration, total sperm count, and motility with corresponding 95% confidence intervals (95% CI). Negative binomial regression was chosen as residuals of main analyses were skewed, and no standard transformations (ie, natural log, cubic) yielded a normal distribution. Participants who reported spillage of the semen sample were excluded from analyses of semen volume and total sperm count. Associations between fetal exposure to smoking and morphology and small testicular volume were calculated with linear and logistic regression, respectively. In all analyses, we tested for interaction between paternal and maternal smoking. If the interaction term showed statistically significant, analyses were stratified into four mutually exclusive groups: no parental smoking; paternal smoking only; maternal smoking only; both parents smoking. If there were no statistically significant interaction, results regarding paternal smoking were adjusted for maternal smoking and vice versa. Covariates for limiting confounding were identified a priori using directed acyclic graph (DAG)[18] and included parental ages at conception, maternal pre-pregnancy BMI, maternal alcohol consumption and caffeine intake in first trimester, and family occupational status—all included in model 1. Sexual abstinence time, clinic, and season all correlate with semen parameters and were included as precision variables in model 2. Analyses with motility as the outcome were further adjusted for time from sample collection to analysis. Maternal smoking during pregnancy is known to increase risk of urogenital and congenital malformations in offspring and such malformations, in particular hypospadias and cryptorchidism, are associated with reduced sperm quality.[19] We therefore ran a final model adjusting for urogenital and congenital malformation to estimate the direct effect of parental smoking on semen parameters not mediated by malformations (model 3). We further performed three sensitivity analyses: In the first, we excluded sons with azoospermia (zero sperm) to assess whether a skewed distribution affected our conclusion and whether potential associations were partly or fully driven by azoospermia (this was only relevant to volume, total sperm count, and semen concentration as azoospermic men were already excluded from previous regressions of motility and morphology). In the second sensitivity analysis, we adjusted for sons own smoking to assess whether a potential association between parental smoking and semen quality is fully or partly mediated by sons own smoking. In the third sensitivity analysis, we excluded all who reported spillage of semen sample as different semen parameters can be affected by whether it is the first or the later fractions that are missing from the sample. The first fractions are more rich in spermatozoa compared to the later fractions.[20] All analyses were performed using STATA-15 (StataCorp, College Station, TX, USA).

2.6 | Ethics

Informed written consent was obtained from all individual participants included in the study. The establishment of the FEPOS cohort was approved by the Scientific Research Ethics Committee for Copenhagen and Frederiksberg (No. H-16015857) and the Danish

Data Protection Agency (No. 2012-58-0004). Moreover, recruitment and data collection were permitted by the Steering Committee of the DNBC (Ref. no. 2016-08).

3 | RESULTS

A total of 772 young men participated in the study. Of these, four were excluded due to missing semen sample and 17 because of missing information on paternal smoking status. Thus, 751 were included for analyses.

The prevalence of paternal smoking was 29% and maternal smoking 22% during first trimester.

Baseline characteristics of the parents and sons according to parents' smoking status are presented in Tables 1 and 2, respectively. No large differences in parental characteristics were observed. However, a higher proportion of sons whose father smoked were current (30%) or occasional smokers (32%) than among sons of non-smoking parents with 26% and 21%, respectively. Days of abstinence were also a little shorter among sons of smoking fathers compared with sons of non-smoking parents (2.2 vs 2.4 days). Time from semen sample collection to analysis was shorter among sons of smoking fathers (46 minutes) relative to sons of non-smoking parents (51 minutes). Spillage of semen sample was reported by 134 participants, and lower among sons of smoking fathers then sons of non-smoking parents (80% vs 82%). Among smoking mothers, 89% reported spillage of semen sample.

3.1 | Semen parameters

Semen parameters according to parents' smoking status are presented in Table 3. In total, 80% of the semen analyses were initiated within one hour of delivery and 99% within 1 hour and 55 minutes. Semen volume, percent progressive, non-progressive, and immotile spermatozoa, percent morphologically normal spermatozoa, and testicular volume did not vary across categories of smoking exposure. However, sons of smoking fathers appeared to have a lower median sperm concentration (39 million/ml) and lower total sperm count (85 million) than sons of non-smoking parents (42 million/ml and 105 million, respectively). The median testicular volume was 15 mL in all exposure groups, but a bigger proportion of sons of smoking fathers had small testicular volume (<15 mL) than sons of non-smoking parents (48% vs 45%).

In Table 4, we present crude and adjusted associations between parental smoking and semen parameters. We found no association between paternal smoking and son's semen volume, motility, and morphology in either crude or adjusted analyses. Analyses regarding semen concentration and total sperm count showed statistically significant interaction terms and were stratified accordingly. In analyses adjusted for confounder and precision variables (model 2), we found that sons of smoking fathers and non-smoking mothers had a 10% (95% CI −24%; 7%) lower semen concentration and 11% (95% CI −27%; 8%) lower total sperm count compared with sons of non-smoking parents. The association between paternal smoking and semen concentration was not modified by maternal smoking but having both a smoking father and mother was associated with a 19% reduction in total sperm count (95% CI −37%; 3%). Further adjustment for urogenital and congenital malformations (model 3) did not seem to explain the observed reduction in semen concentration and total sperm count. Associations were unaffected by exclusion of sons with azoospermia (n = 11, results not shown) and exclusion of sons reporting spillage (n = 134, results not shown), and they were kept in the model. There was no indication that the association was mediated by sons' own smoking (results not shown).

TABLE 1 Baseline characteristics of 751 mothers and fathers according to smoking status in pregnancy

Characteristics	All n = 751	No parental smoking n = 451 [60%]	Paternal smoking only n = 132 [18%]	Maternal smoking only n = 83 [11%]	Both parents smoking n = 85 [11%]
Maternal age at birth in years, mean (SD)	30.4 (4.2)	30.6 (4.0)	31.0 (4.1)	30.1 (4.5)	29.2 (4.8)
Father age at birth in years, mean (SD)	32.8 (5.7)	33.0 (5.8)	33.4 (5.1)	31.8 (5.4)	31.8 (6.2)
Maternal weekly glasses of alcohol, glasses mean (SD)	0.4 (1)	0.4 (1)	0.5 (1)	0.6 (1)	0.4 (1)
Maternal daily coffee consumption, cups mean (SD)	0.9 (2)	0.7 (1)	0.8 (1)	1.4 (3)	1.6 (2)
Pre-pregnancy BMI, mean (SD)	22.8 (3.6)	22.9 (3.6)	22.8 (3.9)	22.5 (3.5)	22.8 (3.5)
Family socioeconomic status, n [%]					
High-grade profession	254 [34]	183 [41]	32 [24]	21 [25]	18 [21]
Low-grade profession	265 [35]	158 [35]	48 [37]	30 [36]	29 [34]
Skilled worker	141 [19]	68 [15]	32 [24]	22 [27]	19 [22]
Unskilled worker	65 [9]	27 [6]	14 [11]	8 [10]	16 [19]
Student	23 [3]	14 [3]	≤5	≤5	≤5
Economically inactive	≤5	≤5	≤5	≤5	≤5

TABLE 2 Baseline characteristics of 751 sons according to parental smoking status

Characteristics	All n = 751	No parental smoking n = 451 [60%]	Paternal smoking only n = 132 [18%]	Maternal smoking only n = 83 [11%]	Both parents smoking n = 85 [11%]
Son BMI, mean (SD)	22.5 (3.4)	22.2 (3.1)	22.7 (3.6)	22.4 (3.6)	23.2 (4.4)
Son smoking habits, n [%]					
Never smoker	357 [48]	236 [53]	51 [39]	43 [52]	27 [32]
Occasional smoker	202 [27]	116 [26]	43 [32]	20 [24]	24 [28]
Current smoker	188 [25]	95 [21]	39 [30]	20 [24]	34 [40]
Son frequency of alcohol consumption, n [%]					
Never/former/less than monthly	88 [12]	46 [10]	20 [15]	11 [13]	11 [13]
1-3 times per month	275 [37]	163 [36]	46 [35]	31 [37]	35 [41]
1-2 times per week	337 [45]	214 [48]	57 [43]	36 [43]	30 [35]
3 or more times per week	47 [6]	24 [5]	9 [7]	≤5	9 [11]
Semen sample collection at the clinic, n [%]	620 [83]	365 [81]	115 [87]	67 [81]	73 [86]
Season of semen sample collection, n [%]					
Winter	151 [20]	90 [20]	23 [17]	19 [23]	19 [22]
Spring	147 [20]	85 [19]	30 [23]	18 [22]	14 [16]
Summer	183 [24]	111 [25]	34 [26]	18 [22]	20 [24]
Fall	270 [36]	165 [37]	45 [34]	28 [34]	32 [38]
Semen sample analysis, Copenhagen n [%]	531 [71]	320 [71]	87 [66]	60 [72]	64 [75]
Minutes from ejaculation to analysis, mean (SD)	50.1 (21.8)	51.0 (22.1)	46.4 (20.2)	48.6 (20.7)	52.0 (23.2)
Days of abstinence, mean (SD)	2.3 (1.4)	2.4 (1.5)	2.2 (1.2)	2.3 (1.5)	2.1 (1.1)
Azoospermia, n [%]	11 [1]	6 [1]	≤5	≤5	≤5
Urogenital malformations, n [%]	118 [16]	62 [14]	20 [15]	16 [19]	20 [24]
Congenital malformations, n [%]	46 [6]	26 [6]	6 [5]	≤5	9 [11]
No spillage of semen sample, n [%]	613 [82]	369 [82]	105 [80]	73 [89]	66 [78]

3.2 | Testicular volume

Analyses suggested a higher risk of small testicular volume (<15 mL) among sons of smoking fathers compared with sons of non-smoking fathers, adjusted for mother's smoking (Odds ratio 1.26, 95% CI 0.89-1.78) (**Table 5**).

4 | DISCUSSION

In this longitudinal study, we found a reduced semen concentration and total sperm count among sons paternally but not maternally exposed to smoking before and during first trimester. Although our statistical model indicated interaction between maternal and paternal smoking, we did not observe an additional reduction in concentration beyond that associated with paternal exposure among sons with two smoking parents but having two smoking parents nearly doubled the reduction in sperm count. We did not find an association between parental smoking and semen volume, number of progressive, non-progressive, immotile, or morphologically normal spermatozoa of the son, but results did indicate a higher risk of small testicular volume among sons of smoking fathers, compared with sons of non-smoking fathers. Sons' own smoking did not explain the observed associations.

Our findings regarding paternal exposure are in-between findings in a number of studies reporting no association with paternal smoking and semen variables or risk of oligospermia[7-10] and findings of large reductions in sperm count and concentration in paternally but not maternally exposed men.[3,11] A general weakness of most of the prior studies—besides being rather small in size[3,7,9-11]—is that the data regarding paternal smoking were retrospectively collected based on the son's or the mother's report long after the relevant pregnancy.[3,7-8,10,11] This implies a risk of misclassification which could have attenuated results to the null. Further, many of the studies lacked information on related parental fetal risk factors to limit potential confounding and could thus have overestimated associations.[3,11] In the present study, the young men were ineligible to participate if they had a history of testicular cancer, chemotherapy, and orchiectomy, or had one or no testicles. These exclusion criteria may have caused us to underestimate the reductions in semen concentration and total sperm count if these men's reproductive health including semen quality is in fact more sensitive to paternal smoking. This could also explain why we observed a smaller reduction in semen concentration and total sperm count as compared to

TABLE 3 Semen quality parameters according to parental smoking status

	All			No parental smoking	Paternal smoking only	Maternal smoking only	Both parents smoking
Sperm parameters	n	Missing	Median (5-95 percentile)	Median (5-95 percentile)	Median (5-95 percentile)	Median (5-95 percentile)	Median (5-95 percentile)
Semen volume, mL[a]	613	138	3 (1-5)	3 (1-5)	3 (1-5)	3 (1-5)	3 (1-5)
Sperm concentration, 10^6 per mL	751	0	38 (3-135)	42 (3-141)	39 (3-119)	35 (5-111)	33 (2-137)
Total sperm count, 10^{6}[a]	613	138	95 (7-401)	105 (10-413)	85 (9-324)	77 (9-260)	70 (5-509)
Progressive spermatozoa, %[b]	740	11	65 (33-84)	65 (34-83)	67 (33-84)	65 (34-85)	64 (27-86)
Non-progressive spermatozoa, %[b]	740	11	6 (1-17)	6 (1-17)	6 (1-16)	5 (1-19)	7 (1-17)
Immotile spermatozoa, %[b]	740	11	28 (13-56)	28 (13-55)	27 (12-55)	28 (14-57)	28 (11-58)
Morphologically normal spermatozoa, %[b,c]	689	62	6 (0-15)	6 (0-15)	7 (1-15)	6 (0-14)	6 (0-16)
Testicular volume, mL	750	1	15 (8-25)	15 (8-25)	15 (7-25)	15 (7-25)	15 (8-23)

[a]134 samples excluded due to spillage.

[b]Motility and morphology not available for 11 azoospermic sons.

[c]Morphological assessments are ongoing.

Axelsson et al (2018) who did not exclude men with history of reproductive disorders.[3,11]

Multiple plausible pathways through which paternal smoking can influence offspring health have been suggested: through passive smoking, through direct exposure of the ovum or embryo via toxic contaminants in the seminal fluid, through DNA damage and by interference with gene expression through imprinting and disruption of the epigenome.[21] In regard to the mechanism of passive smoking, Axelsson et al (2018) did rerun analyses on their first findings[11] on a subsample of 104 sons whose mothers they had cotinine measurements on[3] to see whether the observed reduction in sperm count and concentration could be fully or partly attributed to passive smoking—this only attenuated results slightly indicating this was not the case. We did not have cotinine concentrations available at the time of study. Numerous industrial chemicals can be measured in seminal plasma and can pass the blood-testis barrier.[22] Yet, the actual significance of the direct exposure of the fetus via toxicants in seminal fluid pathway is unknown. Regarding the pathway through DNA damage, several studies have found a higher frequency of chromosomal abnormalities in spermatozoa of smokers and in a recent systematic review based on 200+ studies. Beal et al. (2017) evaluated the evidence of substantial higher rates of DNA damage, mutations, and chromosomal aberrations in spermatozoa of smokers as strong.[6] Lastly, while several studies have demonstrated change in methylation status of somatic cells in smokers,[23] knowledge about methylation and other epigenetic alterations of the sperm epigenome is far less, and no human studies have explicitly demonstrated health effects of paternal pre-conceptional smoking mediated by genetic and/or epigenetic alterations of spermatozoa.[21]

Strengths of our study include the large study population, which is larger than any previous study investigating the association between fetal smoking exposure and semen quality.[3,7-11] Furthermore, data were collected prospectively, and the participants were homogenic according to age and ethnicity. We had the opportunity to adjust for many concurrent fetal risk factors including parental ages, household occupational status, maternal alcohol and caffeine consumption, and pre-pregnancy BMI as well as variables closely linked to semen quality including abstinence time, season, and time from sample delivery to analysis.[24,25] However, as is the case in most similar studies,[3,11] little information on paternal behavior prior to conception was available, and we can therefore not rule out residual confounding.

We only had a single sample from each participant, but although individual semen quality varies slightly in repeated samples, having one sample per participant does not introduce any systematic bias.[26,27]

Our data on parental smoking during pregnancy were based on mothers' self-reports, but we believe the data to be fairly reliable and the resulting risk of misclassification to be low. Self-reported smoking during pregnancy has been found valid in a Norwegian birth cohort.[28] However, if paternal or maternal smoking was misclassified, this would most likely have led to an underestimation of a true association[29] as smokers or ex-smokers are more likely to answer that they did not smoke during pregnancy than non-smokers answering that they did smoke. Although paternal smoking was assessed after conception, we find it reasonable to believe that fathers who smoked during pregnancy also did so before pregnancy. However, we were unable to distinguish never-smoking fathers from former-smoking fathers, and if the association is in fact mediated by DNA damage or epigenetic changes, including ex-smokers in the non-smoking group, would lead to an underestimation of the risk. Ideally, we would have had detailed information on what, how much, and for how long the father had smoked, making necessary dose-response assessments possible. This should be a focal point in future studies.

Our participation rate (18%) was low and implies risk for selection bias, although this is unlikely, as the decision for the young man to participate in the FEPOS cohort should be correlated with both their parents' smoking and with semen quality they do not

TABLE 4 % change in semen quality parameters according to parental smoking status (N = 751)

	Crude	Model 1[a]	Model 2[b]	Model 3[c]
Semen parameters	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
Semen volume, ml[d]				
No paternal smoking	Reference	Reference	Reference	Reference
Paternal smoking	0 (−11, 11)	1 (−10, 12)	2 (−9, 15)	2 (−9, 15)
No maternal smoking	Reference	Reference	Reference	Reference
Maternal smoking	−3 (−14, 9)	−2 (−13, 11)	−1 (−12, 12)	0 (−12, 13)
Semen concentration 106/ml				
No parental smoking	Reference	Reference	Reference	Reference
Paternal smoking	−14 (−28, 2)	−13 (−27, 4)	−10 (−24, 7)	−10 (−24, 7)
Maternal smoking	−23 (−37, −5)	−23 (−38, −4)	−22 (−37, −4)	−21 (−36, −2)
Both parents smoke	−7 (−24, 13)	−9 (−26, 13)	−8 (−25, 13)	−7 (−24, 15)
Total sperm count, 10^{6}[d]				
No parental smoking	Reference	Reference	Reference	Reference
Paternal smoking	−20 (−35, −1)	−17 (−33, 2)	−11 (−27, 8)	−12 (−28, 7)
Maternal smoking	−29 (−44, −10)	−28 (−44, −7)	−26 (−41, −7)	−26 (−41, −8)
Both parents smoke	−11 (−31, 14)	−15 (−35, 11)	−19 (−37, 3)	−18 (−36, 4)
Progressive spermatozoa, %[e]				
No paternal smoking	Reference	Reference	Reference	Reference
Paternal smoking	1 (−6, 8)	0 (−6, 8)	−1 (−8, 6)	−1 (−8, 6)
No maternal smoking	Reference	Reference	Reference	Reference
Maternal smoking	1 (−6, 9)	0 (−8, 8)	0 (−7, 8)	−1 (−8, 7)
Non-progressive spermatozoa, %[e]				
No paternal smoking	Reference	Reference	Reference	Reference
Paternal smoking	−1 (−1, 1)	−1 (−2, 1)	0 (−2, 1)	0 (−2, 1)
No maternal smoking	Reference	Reference	Reference	Reference
Maternal smoking	0 (−2, 2)	0 (−2, 2)	0 (−2, 2)	0 (−2, 2)
Immotile spermatozoa, %[e]				
No paternal smoking	Reference	Reference	Reference	Reference
Paternal smoking	1 (−3, 4)	1 (−3, 4)	1 (−2, 5)	1 (−2, 5)
No maternal smoking	Reference	Reference	Reference	Reference
Maternal smoking	−1 (−4, 3)	0 (−4, 3)	−1 (−4, 3)	−1 (−4, 3)
	Coef. (95% CI)	**Coef. (95% CI)**	**Coef. (95% CI)**	**Coef. (95% CI)**
Morphologically normal sperm, %[f]				
No maternal smoking	Reference	Reference	Reference	Reference
Paternal smoking	0.53 (−0.26, 1.31)	0.40 (−0.42, 1.22)	0.50 (−0.32, 1.32)	0.51 (−0.31, 1.33)
No maternal smoking	Reference	Reference	Reference	Reference
Maternal smoking	−0.26 (−1.12, 0.59)	−0.13 (−1.04, 0.77)	−0.91 (−1.00, 0.81)	−0.06 (−0.97, 0.85)

[a]Model 1: adjusted for parental age at conception, family socioeconomic status, maternal pre-pregnancy BMI, maternal alcohol and coffee consumption, and mutually adjusted when no interaction is found between maternal and paternal smoking.

[b]Model 2: adjusted for variables in model 1+ season, clinic, and sexual abstinence time (analyses of motility also adjusted for time from ejaculation to analysis).

[c]Model 3: adjusted for variables in model 1 and 2+ urogenital and congenital malformations.

[d]Analyses with semen volume and total sperm count exclude 134 sons who reported spillage.

[e]Motility and morphology are not available for 11 azoospermic sons.

[f]Morphological assessments are ongoing.

know. In fertility studies, selection bias, related to outcome, is often caused by the higher propensity for participation by men who are concerned about their fertility and are trying to have children or have previously diagnosed urogenital disorders or suspected infertility.[30-32] We find it, however, unlikely that the participants, due to their age and lack of knowledge about their own

TABLE 5 Risk of small testicular size (<15 ml) according to parental smoking

	Crude	Model 1[a]	Model 2[b]	Model 3[c]
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
No paternal smoking	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Paternal smoking	1.12 (0.81-1.56)	1.21 (0.86-1.70)	1.26 (0.89-1.78)	1.25 (0.88-1.77)
No maternal smoking	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Maternal smoking	0.93 (0.65-1.33)	0.84 (0.58-1.23)	0.85 (0.58-1.24)	0.82 (0.55-1.21)

[a]Model 1: adjusted for parental age at conception, family socioeconomic status, maternal pre-pregnancy BMI, maternal alcohol and coffee consumption, and mutually adjusted when no interaction is found between maternal and paternal smoking.

[b]Model 2: adjusted for variables in model 1 + season and clinic.

[c]Model 3: adjusted for variables in model 1 and 2 + urogenital and congenital malformations.

fertility status, would be able to self-select themselves.[9] The sons are not informed about the specific prenatal exposures of interest, and therefore, the sons' desire to participate is unlikely to depend on paternal smoking.

In conclusion, the study provides limited support for slightly lower sperm concentration, total sperm concentration, and risk of small testicles in sons of smoking fathers, but findings are also compatible with no effect. The study saw no association with semen volume, motility, or morphology.

ACKNOWLEDGEMENTS

We are grateful to all participants and to biomedical laboratory technologists Marianne Lipka Flensborg and Joan Dideriksen for running the clinics and collecting data. We also thank Josefine Rahbæk Larsen for assisting with recruitment and data entry. Furthermore we wish to thank Cecilia Tingsmark for doing the morfology analysis.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Birgit Bjerre Høyer, Ina Olmer Specht, and Jens Peter Bonde contributed to funding acquisition; Katia Keglberg Hærvig, Karin Sørig Hougaard, Cecilia Høst Ramlau-Hansen, Gunnar Toft, Jens Peter Bonde, and Sandra Søgaard Tøttenborg contributed to conceptualization; Katia Keglberg Hærvig, Birgit Bjerre Høyer, Aleksander Giwercman, Cecilia Høst Ramlau-Hansen, Gunnar Toft, Jens Peter Bonde, and Sandra Søgaard Tøttenborg contributed to data acquisition; Katia Keglberg Hærvig contributed to original draft preparation; Katia Keglberg Hærvig and Sandra Søgaard Tøttenborg contributed to analysis and interpretation of results; Katia Keglberg Hærvig, Birgit Bjerre Høyer, Aleksander Giwercman, Karin Sørig Hougaard, Cecilia Høst Ramlau-Hansen, Gunnar Toft, Jens Peter Bonde, and Sandra Søgaard Tøttenborg contributed to critical revision of the manuscript; Katia Keglberg Hærvig, Birgit Bjerre Høyer, Ina Olmer Specht, Aleksander Giwercman, Karin Sørig Hougaard, Cecilia Høst Ramlau-Hansen, Gunnar Toft, Jens Peter Bonde, and Sandra Søgaard Tøttenborg involved in final approval of the manuscript.

ORCID

Katia Keglberg Hærvig https://orcid.org/0000-0002-4250-7948
Aleksander Giwercman https://orcid.org/0000-0001-5816-0785
Gunnar Toft https://orcid.org/0000-0002-7542-6853
Jens Peter Bonde https://orcid.org/0000-0002-8181-3673
Sandra Søgaard Tøttenborg https://orcid.org/0000-0001-7742-4360

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How to cite this article: Hærvig KK, Høyer BB, Giwercman A, et al. Fetal exposure to paternal smoking and semen quality in the adult son. *Andrology*. 2020;8:1117–1125. https://doi.org/10.1111/andr.12782